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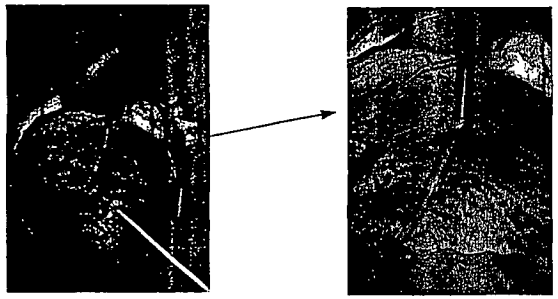

(57) Abstract

Methods and compositions are provided for intra-pericardial administration of anti-microtubule agents, suitable for use in treating or preventing a variety of diseases of the pericardium, heart, or, coronary vasculature.

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INTRAPERICARDIAL DELIVERY OF ANTI-MICROTUBULE AGENTS

TECHNICAL FIELD

The present invention relates generally to compositions, methods and devices for administering anti-microtubule agents into the pericardium.

BACKGROUND OF THE INVENTION

According to U.S. Heart and Stroke Foundation estimates, over 60 million Americans have one or more forms of cardiovascular disease. These diseases claim approximately 1 million lives each year (41% of all deaths in the United States) and are considered the leading cause of death and disability in the developed world.

One such disease, Restenosis, is a form of chronic vascular injury leading to vessel wall thickening and loss of blood flow to the tissue supplied by the blood vessel. It occurs in response to vascular reconstructive procedures, including virtually any manipulation which attempts to relieve vessel obstructions, and is the major factor limiting the effectiveness of invasive treatments for vascular diseases. Restenosis has been a major challenge to cardiovascular research for the past 15 years.

Currently, no existing, FDA approved, treatment for the prevention of restenosis has been completely effective in humans. Systemic therapies that have been investigated include agents directed at treatment of endothelial loss, anti-platelet agents (*e.g.*, aspirin), vasodilators (*e.g.*, calcium channel blockers), antithrombotics (*e.g.*, heparin), anti-inflammatory agents (*e.g.*, steroids), agents which prevent vascular smooth muscle cell (VSMC) proliferation (*e.g.*, colchicine) and promoters of re-endothelialization (*e.g.*, vascular endothelial growth factor). Local treatments which have been investigated include local drug delivery (*e.g.*, heparin) and beta and gamma radiation. All have been disappointing in human use, primarily because they appear to act on a limited portion of the restenotic process. Systemic treatments have also encountered the additional problem of achieving adequate absorption and retention of the drug at the site of the disease to provide a lasting biological effect, without causing unfavorable systemic complications and toxicities.

Balloon angioplasty (with or without stenting) is one of the most widely used treatments for vascular disease; (other options such as laser angioplasty, however, are also available.) While this is the treatment of choice in many cases of severe narrowing of the vasculature, about one-third of patients undergoing balloon angioplasty (source Heart and Stoke Foundation homepage) have renewed narrowing of the treated arteries (restenosis) within 6 months of the initial procedure; often serious enough to necessitate further interventions.

Vascular diseases (including for example, restenosis) are due at least in part to intimal thickening secondary to vascular smooth muscle cell (VSMC) migration, VSMC proliferation and extra-cellular matrix deposition. Briefly, vascular endothelium acts as a nonthrombogenic surface over which blood can flow smoothly and as a barrier which separates the blood components from the tissues comprising the vessel wall. Endothelial cells also release heparin sulphate, prostacyclin, EDRF and other factors that inhibit platelet and white cell adhesion, VSMC contraction, VSMC migration and VSMC proliferation. Any loss or damage to the endothelium, such as occurs during balloon angioplasty, atherectomy, or stent insertion, can result in platelet adhesion, platelet aggregation and thrombus formation. Activated platelets can release substances that produce vasoconstriction (serotonin and thromboxane) and/or promote VSMC migration and proliferation (PDGF, epidermal growth factor, TGF-□ and heparinase). Tissue factors released by the arteries stimulates clot formation resulting in a fibrin matrix into which smooth muscle cells can migrate and proliferate.

This cascade of events leads to the transformation of vascular smooth muscle cells from a contractile to a secretory phenotype. Angioplasty induced cell lysis and matrix destruction results in local release of basic fibroblast growth factor (bFGF) which in turn stimulates VSMC proliferation directly and indirectly through the induction of PDGF production. In addition to PDGF and bFGF, VSMC proliferation is also stimulated by platelet released EGF and insulin growth factor-1.

Vascular smooth muscle cells are also induced to migrate into the media and intima of the vessel. This is enabled by release and activation of matrix metalloproteases which degrade a pathway for the VSMC through the extra-cellular matrix and internal elastic lamina of the vessel wall. After migration and proliferation the vascular smooth muscle cells then deposit an extra-cellular matrix consisting of gylcosaminoglycans, elastin and collagen

which comprises the largest part of intimal thickening. A significant portion of the restenosis process may be due to remodeling of the vascular wall leading to changes in the overall size of the artery; at least some of which is secondary to proliferation within the adventitia (in addition to the media). The net result of these processes is a recurrence of the narrowing of the vascular wall which is often severe enough to require a repeat intervention.

The present invention provides compositions and methods for intrapericardially delivering an anti-microtubule agent so that disease within the pericardium, heart, or coronary vasculature (*e.g.*, restenosis, primary stenosis, or, atherosclerosis) may be treated or prevented. These compositions and methods address the problems associated with the existing procedures, offer significant advantages when compared to existing procedures, and further provides other, related advantages.

SUMMARY OF THE INVENTION

Briefly stated, the present invention provides methods for administering intrapericardially an anti-microtubule agent, suitable for treating or preventing disease of the pericardium, heart, or, coronary vasculature. The anti-microtubule agent is administered into the pericardial sac (*i.e.*, the anatomical space between the two layers of the pericardium) via a specialized needle placed through the chest wall, through the myocardium, or through the vessel wall of the vasculature supplying the heart, with or without radiographic guidance. Similarly, the agent can be placed in the pericardial cavity under direct vision during open or endoscopic surgical procedures. The anti-microtubule is present at the disease site in sufficient doses to favorably impact on cardiac conditions such as coronary stenosis, restenosis, in-stent restenosis, and atherosclerosis; surgical adhesions following cardiac surgery (open, endoscopic, or catheter –based); stenosis or failure of valve replacement surgery; accelerated atherosclerosis following cardiac transplantation; immunological rejection following cardiac transplantation (host vs. graft disease); cardiac rhythm abnormalities; rheumatic or inflammatory diseases with cardiac manifestations (*i.e.*, rheumatoid arthritis, systemic lupus erythematosis, vasculitis); infections of the myocardium or surrounding tissues; and/or primary or metastatic malignancy of the myocarium, pericardium *e.g.*, malignant pericardial effussion), or surrounding tissues. Local administration of the agent to the pericardial sac can increase the efficacy of the agent by

increasing the local dose of the agent while decreasing the systemic absorption and potential toxicity of the agent.

Representative examples of such agents include taxanes (*e.g.*, paclitaxel and docetaxel), eleutherobin, sarcodictyins, epothilones A and B, discodermolide, deuterium oxide (D_2O), hexylene glycol (2-methyl-2,4-pentanediol), tubercidin (7-deazaadenosine), LY290181 (2-amino-4-(3-pyridyl)-4H-naphtho(1,2-b)pyran-3-cardonitrile), aluminum fluoride, ethylene glycol bis-(succinimidylsuccinate), glycine ethyl ester, nocodazole, cytochalasin B, colchicine, colcemid, podophyllotoxin, benomyl, oryzalin, majusculamide C, demecolcine, methyl-2-benzimidazolecarbamate (MBC), LY195448, subtilisin, 1069C85, steganacin, combretastatin, curacin, estradiol, 2-methoxyestradiol, flavanol, rotenone, griseofulvin, vinca alkaloids, including vinblastine and vincristine, maytansinoids and ansamitocins, rhizoxin, phomopsin A, ustiloxins, dolastatin 10, dolastatin 15, halichondrins and halistatins, spongistatins, cryptophycins, rhazinilam, betaine, taurine, isethionate, HO-221, adociasulfate-2, estramustine, monoclonal anti-idiotypic antibodies, microtubule assembly promoting protein (taxol-like protein, TALP), cell swelling induced by hypotonic (190 mosmol/L) conditions, insulin (100 nmol/L) or glutamine (10 mmol/L), dynein binding, gibberelin, XCHO1 (kinesin-like protein), lysophosphatidic acid, lithium ion, plant cell wall components (*e.g.*, poly-L-lysine and extensin), glycerol buffers, Triton X-100 microtubule stabilizing buffer, microtubule associated proteins (*e.g.*, MAP2, MAP4, tau, big tau, ensconsin, elongation factor-1-alpha (EF-1α) and E-MAP-115), cellular entities (*e.g.*, histone H1, myelin basic protein and kinetochores), endogenous microtubular structures (*e.g.*, axonemal structures, plugs and GTP caps), stable tubule only polypeptide (*e.g.*, STOP145 and STOP220) and tension from mitotic forces, as well as any analogues and derivatives of any of the above. .

In certain embodiments, the anti-microtubule agent is formulated suitable to provide prolonged release of the agent at the site of administration, localize the agent to a specific site of administration, reduce the trauma and subsequent scarring associated with the procedure, or make the agent suitable for injection or surgical placement within the pericardial sac. The anti-microtubule agents may be formulated along with other compounds or compositions, such as, for example, an ointment, cream, lotion, gel, spray, foam, mousse,

coating, wrap, paste, barrier, implant, microsphere, microparticle, film or the like. Within certain embodiments, the compound or composition may function as a carrier, which may be either polymeric, or non-polymeric. Representative examples of polymeric carriers include poly(ethylene-vinyl acetate), copolymers of lactic acid and glycolic acid, poly (caprolactone), poly (lactic acid), copolymers of poly (lactic acid) and poly (caprolactone), gelatin, hyaluronic acid, collagen matrices, celluloses and albumen. Representative examples of other suitable carriers include, but are not limited to ethanol; mixtures of ethanol and glycols (*e.g.*, ethylene glycol or propylene glycol); mixtures of ethanol and isopropyl myristate or ethanol, isopropyl myristate and water (*e.g.*, 55:5:40); mixtures of ethanol and eineol or D-limonene (with or without water); glycols (*e.g.*, ethylene glycol or propylene glycol) and mixtures of glycols such as propylene glycol and water, phosphatidyl glycerol, dioleoylphosphatidyl glycerol, Transcutol®, or terpinolene; mixtures of isopropyl myristate and 1-hexyl-2-pyrrolidone, N-dodecyl-2-piperidinone or 1-hexyl-2-pyrrolidone.

These and other aspects of the present invention will become evident upon reference to the following detailed description and attached drawings. In addition, various references are set forth below which describe in more detail certain procedures, devices or compositions, and are therefore incorporated by reference in their entirety.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graphic illustration of one representative animal model which utilizes balloon injury of the LAD or LC.

Figure 2 is a bar graph which shows fracture length 28 days following balloon injury.

Figure 3 is a bar graph which shows the percentage change in diameter following balloon injury and treatment with control micelles, or, 50 mg or 10 mg paclitaxel-loaded micelles.

Figure 4 provides 2 photographs which depict swine coronary arteries following balloon injury and treatment with control micelles, or, 50 mg paclitaxel loaded micelles.

Figure 5 is a bar graph which shows neoinimal area/fracture length.

Figure 6 is a bar graph which shows the lumen diameter following balloon injury and treatment with control micelles, or, 50 mg or 10 mg paclitaxel-loaded micelles.

Figure 7 is a graph which shows the effect of IPC delivery of paclitaxel on vessel response to overstretch injury. The R^2 values for the linear regression analyses of control, HD and LD were 0.69, 0.66 and 0.44, respectively. A positive effect is presented by treated groups, which show bigger fracture length, smaller neointimal area.

Figures 8A and 8B are bar graphs which show the results of morphometric data of IPC delivery of paclitaxel after 28 days. (A) and (B) respectively depict the neointimal area and percent stenosis for each of the three groups. * indicates the values of $p < .001$ vs control.

Figures 9A and 9B are photomicrographs of two AV fistulae created in the same pig: (A) fistula treated with 5% paclitaxel EVA film and (B) not treated. Note the pronounced venous intimal thickening in the non-treated fistula (B) and the complete inhibition of intimal hyperplasia in the treated fistula (A). Also note the periadventitial fibrin layer in the treated fistula (A).

Figures 10A and 10B are high magnification photomicrographs at the anastomosis of two AV fistulae created in the same pig: (A) fistula treated with 20% paclitaxel EVA film and (B) non-treated. Note the marked venous intimal hyperplasia in the non-treated fistula (B) and the absence of intimal hyperplasia in the treated fistula (A). Also note the fibrin layer in the treated fistula.

DETAILED DESCRIPTION OF THE INVENTION

Prior to setting forth the invention, it may be helpful to an understanding thereof to set forth definitions of certain terms that will be used hereinafter.

"Anti-microtubule Agent" should be understood to include any protein, peptide, chemical, or other molecule which impairs the function of microtubules, for example, through the prevention or stabilization of polymerization. A wide variety of methods may be utilized to determine the anti-microtubule activity of a particular compound, including for example, assays described by Smith et al. (*Cancer Lett* 79(2):213-219, 1994) and Mooberry et al., (*Cancer Lett.* 96(2):261-266, 1995).

As noted above, the present invention provides methods for treating or preventing disease of the pericardium, heart, or coronary vasculature, comprising the step of administering to the pericardium, heart or, coronary vasculature an anti-microtubule agent. The anti-microtubule is presented to the disease site in sufficient doses to favorably impact on cardiac conditions such as coronary stenosis, restenosis, in-stent restenosis, and atherosclerosis; surgical adhesions following cardiac surgery (open, endoscopic, or catheter-based); stenosis or failure of valve replacement surgery; restenosis following cardiac shunt procedures; accelerated atherosclerosis following cardiac transplantation; immunological rejection following cardiac transplantation (host vs. graft disease); cardiac rhythm abnormalities; rheumatic or inflammatory diseases with cardiac manifestations (*i.e.*, rheumatoid arthritis, systemic lupus erythematosis, vasculitis); infections of the myocardium or surrounding tissues; and/or primary or metastatic malignancy of the myocarium, pericardium *e.g.*, malignant pericardial effussion), or surrounding tissues.

Briefly, a wide variety of anti-microtubule agents may be delivered, either with or without a carrier (*e.g.*, a polymer or ointment), in order to treat or prevent disease. Representative examples of such agents include taxanes (*e.g.*, paclitaxel (discussed in more detail below) and docetaxel) (Schiff et al., *Nature 277*:665-667, 1979; Long and Fairchild, *Cancer Research 54*: 4355-4361, 1994; Ringel and Horwitz, *J. Natl. Cancer Inst. 83*(4):288-291, 1991; Pazdur et al., *Cancer Treat. Rev. 19*(4):351-386, 1993), eleutherobin (*e.g.*, U.S. Patent No. 5,473,057), sarcodictyins (including sarcodictyin A), epothilones A and B (Bollag et al., *Cancer Research 55*:2325-2333, 1995), discodermolide (ter Haar et al., *Biochemistry 35*:243-250, 1996), deuterium oxide (D_2O) (James and Lefebvre, *Genetics 130*(2):305-314, 1992; Sollott et al., *J. Clin. Invest. 95*:1869-1876, 1995), hexylene glycol (2-methyl-2,4-pentanediol) (Oka et al., *Cell Struct. Funct. 16*(2):125-134, 1991), tubercidin (7-deazaadenosine) (Mooberry et al., *Cancer Lett. 96*(2):261-266, 1995), LY290181 (2-amino-4-(3-pyridyl)-4H-naphtho(1,2-b)pyran-3-cardonitrile) (Panda et al., *J. Biol. Chem. 272*(12):7681-7687, 1997; Wood et al., *Mol. Pharmacol. 52*(3):437-444, 1997), aluminum fluoride (Song et al., *J. Cell. Sci. Suppl. 14*:147-150, 1991), ethylene glycol bis-(succinimidylsuccinate) (Caplow and Shanks, *J. Biol. Chem. 265*(15):8935-8941, 1990), glycine ethyl ester (Mejillano et al., *Biochemistry 31*(13):3478-3483, 1992), nocodazole

(Ding et al., *J. Exp. Med. 171*(3):715-727, 1990; Dotti et al., *J. Cell Sci. Suppl. 15*:75-84, 1991; Oka et al., *Cell Struct. Funct. 16*(2):125-134, 1991; Weimer et al., *J. Cell. Biol. 136*(1):71-80, 1997), cytochalasin B (Illinger et al., *Biol. Cell 73*(2-3):131-138, 1991), colchicine and CI 980 (Allen et al., *Am. J. Physiol. 261*(4 Pt. 1):L315-L321, 1991; Ding et al., *J. Exp. Med. 171*(3): 715-727, 1990; Gonzalez et al., *Exp. Cell. Res. 192*(1):10-15, 1991; Stargell et al., *Mol. Cell. Biol. 12*(4):1443-1450, 1992; Garcia et al., *Antican. Drugs 6*(4):533-544, 1995), colcemid (Barlow et al., *Cell. Motil. Cytoskeleton 19*(1):9-17, 1991; Meschini et al., *J. Microsc. 176*(Pt. 3): 204-210, 1994; Oka et al., *Cell Struct. Funct. 16*(2):125-134, 1991), podophyllotoxin (Ding et al., *J. Exp. Med. 171*(3):715-727, 1990), benomyl (Hardwick et al., *J. Cell. Biol. 131*(3):709-720, 1995; Shero et al., *Genes Dev.* 5(4): 549-560, 1991), oryzalin (Stargell et al., *Mol. Cell. Biol.* 12(4): 1443-1450, 1992), majusculamide C (Moore, *J. Ind. Microbiol.* 16(2): 134-143, 1996), demecolcine (Van Dolah and Ramsdell, *J. Cell. Physiol.* 166(1): 49-56, 1996; Wiemer et al., *J. Cell. Biol.* 136(1): 71-80, 1997), methyl-2-benzimidazolecarbamate (MBC) (Brown et al., *J. Cell. Biol.* 123(2): 387-403, 1993), LY195448 (Barlow & Cabral, *Cell Motil. Cytoskel.* 19: 9-17, 1991), subtilisin (Saoudi et al., *J. Cell Sci.* 108: 357-367, 1995), 1069C85 (Raynaud et al., *Cancer Chemother. Pharmacol.* 35: 169-173, 1994), steganacin (Hamel, *Med. Res. Rev. 16*(2):207-231, 1996), combretastatins (Hamel, *Med. Res. Rev.* 16(2):207-231, 1996), curacins (Hamel, *Med. Res. Rev.* 16(2): 207-231, 1996), estradiol (Aizu-Yokata et al., *Carcinogen.* 15(9):1875-1879, 1994), 2-methoxyestradiol (Hamel, *Med. Res. Rev.* 16(2):207-231, 1996), flavanols (Hamel, *Med. Res. Rev.* 16(2):207-231, 1996), rotenone (Hamel, *Med. Res. Rev.* 16(2):207-231, 1996), griseofulvin (Hamel, *Med. Res. Rev.* 16(2):207-231, 1996), vinca alkaloids, including vinblastine and vincristine (Ding et al., *J. Exp. Med.* 171(3):715-727, 1990; Dirk et al., *Neurochem. Res.* 15(11):1135-1139, 1990; Hamel, *Med. Res. Rev.* 16(2):207-231, 1996; Illinger et al., *Biol. Cell* 73(2-3):131-138, 1991; Wiemer et al., *J. Cell. Biol.* 136(1):71-80, 1997), maytansinoids and ansamitocins (Hamel, *Med. Res. Rev.* 16(2):207-231, 1996), rhizoxin (Hamel, *Med. Res. Rev.* 16(2):207-231, 1996), phomopsin A (Hamel, *Med. Res. Rev.* 16(2):207-231, 1996), ustiloxins (Hamel, *Med. Res. Rev.* 16(2):207-231, 1996), dolastatin 10 (Hamel, *Med. Res. Rev.* 16(2):207-231, 1996), dolastatin 15 (Hamel, *Med. Res. Rev.* 16(2):207-231, 1996), halichondrins and halistatins (Hamel, *Med. Res. Rev.* 16(2):207-231,

1996), spongistatins (Hamel, *Med. Res. Rev.* 16(2):207-231, 1996), cryptophycins (Hamel, *Med. Res. Rev.* 16(2):207-231, 1996), rhazinilam (Hamel, *Med. Res. Rev.* 16(2):207-231, 1996), betaine (Hashimoto et al., *Zool. Sci.* 1:195-204, 1984), taurine (Hashimoto et al., *Zool. Sci.* 1:195-204, 1984), isethionate (Hashimoto et al., *Zool. Sci.* 1:195-204, 1984), HO-221 (Ando et al., *Cancer Chemother. Pharmacol.* 37:63-69, 1995), adociasulfate-2 (Sakowicz et al., *Science* 280:292-295, 1998), estramustine (Panda et al., *Proc. Natl. Acad. Sci. USA* 94:10560-10564, 1997), monoclonal anti-idiotypic antibodies (Leu et al., *Proc. Natl. Acad. Sci. USA* 91(22):10690-10694, 1994), microtubule assembly promoting protein (taxol-like protein, TALP) (Hwang et al., *Biochem. Biophys. Res. Commun.* 208(3):1174-1180, 1995), cell swelling induced by hypotonic (190 mosmol/L) conditions, insulin (100 nmol/L) or glutamine (10 mmol/L) (Haussinger et al., *Biochem. Cell. Biol.* 72(1-2):12-19, 1994), dynein binding (Ohba et al., *Biochim. Biophys. Acta* 1158(3):323-332, 1993), gibberelin (Mita and Shibaoka, *Protoplasma* 119(1/2):100-109, 1984), XCHO1 (kinesin-like protein) (Yonetani et al., *Mol. Biol. Cell* 7(suppl):211A, 1996), lysophosphatidic acid (Cook et al., *Mol. Biol. Cell* 6(suppl):260A, 1995), lithium ion (Bhattacharyya and Wolff, *Biochem. Biophys. Res. Commun.* 73(2):383-390, 1976), plant cell wall components (*e.g.*, poly-L-lysine and extensin) (Akashi et al., *Planta* 182(3):363-369, 1990), glycerol buffers (Schilstra et al., *Biochem. J.* 277(Pt. 3):839-847, 1991; Farrell and Keates, *Biochem. Cell. Biol.* 68(11): 1256-1261, 1990; Lopez et al., *J. Cell. Biochem.* 43(3): 281-291, 1990), Triton X-100 microtubule stabilizing buffer (Brown et al., *J. Cell Sci.* 104(Pt. 2): 339-352, 1993; Safiejko-Mroczka and Bell, *J. Histochem. Cytochem.* 44(6): 641-656, 1996), microtubule associated proteins (*e.g.*, MAP2, MAP4, tau, big tau, ensconsin, elongation factor-1-alpha (EF-1α) and E-MAP-115) (Burgess et al., *Cell Motil. Cytoskeleton* 20(4): 289-300, 1991; Saoudi et al., *J. Cell. Sci.* 108(Pt. 1): 357-367, 1995; Bulinski and Bossler, *J. Cell. Sci.* 107(Pt. 10): 2839-2849, 1994; Ookata et al., *J. Cell Biol.* 128(5): 849-862, 1995; Boyne et al., *J. Comp. Neurol.* 358(2): 279-293, 1995; Ferreira and Caceres, *J. Neurosci.* 11(2): 392-400, 1991; Thurston et al., *Chromosoma* 105(1): 20-30, 1996; Wang et al., *Brain Res. Mol. Brain Res.* 38(2): 200-208, 1996; Moore and Cyr, *Mol. Biol. Cell* 7(suppl): 221-A, 1996; Masson and Kreis, *J. Cell Biol.* 123(2), 357-371, 1993), cellular entities (*e.g.*, histone H1, myelin basic protein and kinetochores) (Saoudi et al., *J. Cell. Sci.* 108(Pt. 1): 357-367, 1995; Simerly et al., *J. Cell Biol.* 111(4): 1491-1504,

1990), endogenous microtubular structures (*e.g.*, axonemal structures, plugs and GTP caps) (Dye et al., *Cell Motil. Cytoskeleton* 21(3): 171-186, 1992; Azhar and Murphy, *Cell Motil. Cytoskeleton* 15(3): 156-161, 1990; Walker et al., *J. Cell Biol.* 114(1): 73-81, 1991; Drechsel and Kirschner, *Curr. Biol.* 4(12): 1053-1061, 1994), stable tubule only polypeptide (*e.g.*, STOP145 and STOP220) (Pirollet et al., *Biochim. Biophys. Acta* 1160(1): 113-119, 1992; Pirollet et al., *Biochemistry* 31(37): 8849-8855, 1992; Bosc et al., *Proc. Natl. Acad. Sci. USA* 93(5): 2125-2130, 1996; Margolis et al., *EMBO J.* 9(12): 4095-4102, 1990) and tension from mitotic forces (Nicklas and Ward, *J. Cell Biol.* 126(5): 1241-1253, 1994), as well as any analogues and derivatives of any of the above. Such compounds can act by either depolymerizing microtubules (*e.g.*, colchicine and vinblastine), or by stabilizing microtubule formation (*e.g.*, paclitaxel).

Within one preferred embodiment of the invention, the therapeutic agent is paclitaxel, a compound which disrupts microtubule formation by binding to tubulin to form abnormal mitotic spindles. Briefly, paclitaxel is a highly derivatized diterpenoid (Wani et al., *J. Am. Chem. Soc. 93*:2325, 1971) which has been obtained from the harvested and dried bark of *Taxus brevifolia* (Pacific Yew) and *Taxomyces Andreanae* and *Endophytic Fungus* of the Pacific Yew (Stierle et al., *Science 60*:214-216, 1993). "Paclitaxel" (which should be understood herein to include prodrugs, analogues and derivatives such as, for example, TAXOL®, TAXOTERE®, Docetaxel, 10-desacetyl analogues of paclitaxel and 3'N-desbenzoyl-3'N-t-butoxy carbonyl analogues of paclitaxel) may be readily prepared utilizing techniques known to those skilled in the art (*see e.g.*, Schiff et al., *Nature 277*:665-667, 1979; Long and Fairchild, *Cancer Research 54*:4355-4361, 1994; Ringel and Horwitz, *J. Natl. Cancer Inst. 83*(4):288-291, 1991; Pazdur et al., *Cancer Treat. Rev. 19*(4):351-386, 1993; WO 94/07882; WO 94/07881; WO 94/07880; WO 94/07876; WO 93/23555; WO 93/10076; WO94/00156; WO 93/24476; EP 590267; WO 94/20089; U.S. Patent Nos. 5,294,637; 5,283,253; 5,279,949; 5,274,137; 5,202,448; 5,200,534; 5,229,529; 5,254,580; 5,412,092; 5,395,850; 5,380,751; 5,350,866; 4,857,653; 5,272,171; 5,411,984; 5,248,796; 5,248,796; 5,422,364; 5,300,638; 5,294,637; 5,362,831; 5,440,056; 4,814,470; 5,278,324; 5,352,805; 5,411,984; 5,059,699; 4,942,184; *Tetrahedron Letters 35*(52):9709-9712, 1994; *J. Med. Chem. 35*:4230-4237, 1992; *J. Med. Chem. 34*:992-998, 1991; *J. Natural Prod. 57*(10):1404-

1410, 1994; *J. Natural Prod.* 57(11):1580-1583, 1994; *J. Am. Chem. Soc.* 110:6558-6560, 1988), or obtained from a variety of commercial sources, including for example, Sigma Chemical Co., St. Louis, Missouri (T7402 - from *Taxus brevifolia*).

Representative examples of such paclitaxel derivatives or analogues include 7-deoxy-docetaxol, 7,8-cyclopropataxanes, N-substituted 2-azetidones, 6,7-epoxy paclitaxels, 6,7-modified paclitaxels, 10-desacetoxytaxol, 10-deacetyltaxol (from 10-deacetylbaccatin III), phosphonooxy and carbonate derivatives of taxol, taxol 2',7-di(sodium 1,2-benzenedicarboxylate, 10-desacetoxy-11,12-dihydrotaxol-10,12(18)-diene derivatives, 10-desacetoxytaxol, Protaxol (2'-and/or 7-O-ester derivatives), (2'-and/or 7-O-carbonate derivatives), asymmetric synthesis of taxol side chain, fluoro taxols, 9-deoxotaxane, (13-acetyl-9-deoxobaccatine III, 9-deoxotaxol, 7-deoxy-9-deoxotaxol, 10-desacetoxy-7-deoxy-9-deoxotaxol, Derivatives containing hydrogen or acetyl group and a hydroxy and tert-butoxycarbonylamino, sulfonated 2'-acryloyltaxol and sulfonated 2'-O-acyl acid taxol derivatives, succinyltaxol, 2'-γ-aminobutyryltaxol formate, 2'-acetyl taxol, 7-acetyl taxol, 7-glycine carbamate taxol, 2'-OH-7-PEG(5000) carbamate taxol, 2'-benzoyl and 2',7-dibenzoyl taxol derivatives, other prodrugs (2'-acetyltaxol; 2',7-diacetyltaxol; 2'succinyltaxol; 2'-(beta-alanyl)-taxol); 2'gamma-aminobutyryltaxol formate; ethylene glycol derivatives of 2'-succinyltaxol; 2'-glutaryltaxol; 2'-(N,N-dimethylglycyl) taxol; 2'-(2-(N,N-dimethylamino)propionyl)taxol; 2'orthocarboxybenzoyl taxol; 2'aliphatic carboxylic acid derivatives of taxol, Prodrugs {2'(N,N-diethylaminopropionyl)taxol, 2'(N,N-dimethylglycyl)taxol, 7(N,N-dimethylglycyl)taxol, 2',7-di-(N,N-dimethylglycyl)taxol, 7(N,N-diethylaminopropionyl)taxol, 2',7-di(N,N-diethylaminopropionyl)taxol, 2'-(L-glycyl)taxol, 7-(L-glycyl)taxol, 2',7-di(L-glycyl)taxol, 2'-(L-alanyl)taxol, 7-(L-alanyl)taxol, 2',7-di(L-alanyl)taxol, 2'-(L-leucyl)taxol, 7-(L-leucyl)taxol, 2',7-di(L-leucyl)taxol, 2'-(L-isoleucyl)taxol, 7-(L-isoleucyl)taxol, 2',7-di(L-isoleucyl)taxol, 2'-(L-valyl)taxol, 7-(L-valyl)taxol, 2'7-di(L-valyl)taxol, 2'-(L-phenylalanyl)taxol, 7-(L-phenylalanyl)taxol, 2',7-di(L-phenylalanyl)taxol, 2'-(L-prolyl)taxol, 7-(L-prolyl)taxol, 2',7-di(L-prolyl)taxol, 2'-(L-lysyl)taxol, 7-(L-lysyl)taxol, 2',7-di(L-lysyl)taxol, 2'-(L-glutamyl)taxol, 7-(L-glutamyl)taxol, 2',7-di(L-glutamyl)taxol, 2'-(L-arginyl)taxol, 7-(L-arginyl)taxol, 2',7-di(L-arginyl)taxol}, Taxol analogs with modified phenylisoserine side chains, taxotere, (N-debenzoyl-N-tert-

(butoxycaronyl)-10-deacetyltaxol, and taxanes (*e.g.*, baccatin III, cephalomannine, 10-deacetylbaccatin III, brevifoliol, yunantaxusin and taxusin).

FORMULATIONS

As noted above, therapeutic anti-microtubule agents described herein may be formulated in a variety of manners, and thus may additionally comprise a carrier. In this regard, a wide variety of carriers may be selected of either polymeric or non-polymeric origin.

For example, within one embodiment of the invention a wide variety of polymeric carriers may be utilized to contain and/or deliver one or more of the therapeutic agents discussed above, including for example both biodegradable and non-biodegradable compositions. Representative examples of biodegradable compositions include albumin, collagen, gelatin, hyaluronic acid, starch, cellulose (methylcellulose, hydroxypropylcellulose, hydroxypropylmethylcellulose,, hydroxyethylcellulose, carboxymethylcellulose, cellulose acetate phthalate, cellulose acetate succinate, hydroxypropylmethylcellulose phthalate), casein, dextrans, polysaccharides, fibrinogen, poly(D,L lactide), poly(D,L-lactide-co-glycolide), poly(glycolide), poly(hydroxybutyrate), poly(alkylcarbonate) and poly(orthoesters), polyesters, poly(hydroxyvaleric acid), polydioxanone, poly(ethylene terephthalate), poly(malic acid), poly(tartronic acid), polyanhydrides, polyphosphazenes, poly(amino acids) and their copolymers (*see generally*, Illum, L., Davids, S.S. (eds.) "Polymers in Controlled Drug Delivery" Wright, Bristol, 1987; Arshady, *J. Controlled Release* 17:1-22, 1991; Pitt, *Int. J. Phar.* 59:173-196, 1990; Holland et al., *J. Controlled Release* 4:155-0180, 1986). Representative examples of nondegradable polymers include poly(ethylene-vinyl acetate) ("EVA") copolymers, silicone rubber, acrylic polymers (polyacrylic acid, polymethylacrylic acid, polymethylmethacrylate, polyalkylcynoacrylate), polyethylene, polyproplene, polyamides (nylon 6,6), polyurethane, poly(ester urethanes), poly(ether urethanes), poly(ester-urea), polyethers (poly(ethylene oxide), poly(propylene oxide), Pluronics and poly(tetramethylene glycol)), silicone rubbers and vinyl polymers (polyvinylpyrrolidone, poly(vinyl alcohol), poly(vinyl acetate phthalate). Polymers may also be developed which are either anionic (*e.g.*, alginate, carrageenin, carboxymethyl cellulose and poly(acrylic acid), or cationic (*e.g.*, chitosan, poly-L-lysine, polyethylenimine, and poly (allyl amine)) (*see generally*, Dunn et al., *J. Applied Polymer Sci.* 50:353-365, 1993; Cascone

et al., *J. Materials Sci.: Materials in Medicine 5*:770-774, 1994; Shiraishi et al., *Biol. Pharm. Bull. 16*(11):1164-1168, 1993; Thacharodi and Rao, *Int'l J. Pharm. 120*:115-118, 1995; Miyazaki et al., *Int'l J. Pharm. 118*:257-263, 1995). Particularly preferred polymeric carriers include poly(ethylene-vinyl acetate), poly (D,L-lactic acid) oligomers and polymers, poly (L-lactic acid) oligomers and polymers, poly (glycolic acid), copolymers of lactic acid and glycolic acid, poly (caprolactone), poly (valerolactone), polyanhydrides, copolymers of poly (caprolactone) or poly (lactic acid) with a polyethylene glycol (*e.g.*, MePEG), and blends thereof.

Polymeric carriers can be fashioned in a variety of forms, with desired release characteristics and/or with specific desired properties. For example, polymeric carriers may be fashioned to release a therapeutic agent upon exposure to a specific triggering event such as pH (*see e.g.*, Heller et al., "Chemically Self-Regulated Drug Delivery Systems," in *Polymers in Medicine III*, Elsevier Science Publishers B.V., Amsterdam, 1988, pp. 175-188; Kang et al., *J. Applied Polymer Sci. 48*:343-354, 1993; Dong et al., *J. Controlled Release 19:171-178, 1992;* Dong and Hoffman, *J. Controlled Release 15*:141-152, 1991; Kim et al., *J. Controlled Release 28*:143-152, 1994; Cornejo-Bravo et al., *J. Controlled Release 33*:223-229, 1995; Wu and Lee, *Pharm. Res. 10*(10):1544-1547, 1993; Serres et al., *Pharm. Res. 13*(2):196-201, 1996; Peppas, "Fundamentals of pH- and Temperature-Sensitive Delivery Systems," in Gurny et al. (eds.), *Pulsatile Drug Delivery*, Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart, 1993, pp. 41-55; Doelker, "Cellulose Derivatives," 1993, in Peppas and Langer (eds.), *Biopolymers I*, Springer-Verlag, Berlin). Representative examples of pH-sensitive polymers include poly(acrylic acid) and its derivatives (including for example, homopolymers such as poly(aminocarboxylic acid); poly(acrylic acid); poly(methyl acrylic acid), copolymers of such homopolymers, and copolymers of poly(acrylic acid) and acrylmonomers such as those discussed above. Other pH sensitive polymers include polysaccharides such as cellulose acetate phthalate; hydroxypropylmethylcellulose phthalate; hydroxypropylmethylcellulose acetate succinate; cellulose acetate trimellilate; and chitosan. Yet other pH sensitive polymers include any mixture of a pH sensitive polymer and a water soluble polymer.

Likewise, polymeric carriers can be fashioned which are temperature sensitive (*see e.g.*, Chen et al., "Novel Hydrogels of a Temperature-Sensitive Pluronic Grafted to a Bioadhesive Polyacrylic Acid Backbone for Vaginal Drug Delivery," in *Proceed. Intern. Symp. Control. Rel. Bioact. Mater. 22*:167-168, Controlled Release Society, Inc., 1995; Okano, "Molecular Design of Stimuli-Responsive Hydrogels for Temporal Controlled Drug Delivery," in *Proceed. Intern. Symp. Control. Rel. Bioact. Mater. 22*:111-112, Controlled Release Society, Inc., 1995; Johnston et al., *Pharm. Res. 9*(3):425-433, 1992; Tung, *Int'l J. Pharm. 107*:85-90, 1994; Harsh and Gehrke, *J. Controlled Release 17*:175-186, 1991; Bae et al., *Pharm. Res. 8*(4):531-537, 1991; Dinarvand and D'Emanuele, *J. Controlled Release 36*:221-227, 1995; Yu and Grainger, "Novel Thermo-sensitive Amphiphilic Gels: Poly N-isopropylacrylamide-co-sodium acrylate-co-n-N-alkylacrylamide Network Synthesis and Physicochemical Characterization," Dept. of Chemical & Biological Sci., Oregon Graduate Institute of Science & Technology, Beaverton, OR, pp. 820-821; Zhou and Smid, "Physical Hydrogels of Associative Star Polymers," Polymer Research Institute, Dept. of Chemistry, College of Environmental Science and Forestry, State Univ. of New York, Syracuse, NY, pp. 822-823; Hoffman et al., "Characterizing Pore Sizes and Water 'Structure' in Stimuli-Responsive Hydrogels," Center for Bioengineering, Univ. of Washington, Seattle, WA, p. 828; Yu and Grainger, "Thermo-sensitive Swelling Behavior in Crosslinked N-isopropylacrylamide Networks: Cationic, Anionic and Ampholytic Hydrogels," Dept. of Chemical & Biological Sci., Oregon Graduate Institute of Science & Technology, Beaverton, OR, pp. 829-830; Kim et al., *Pharm. Res. 9*(3):283-290, 1992; Bae et al., *Pharm. Res. 8*(5):624-628, 1991; Kono et al., *J. Controlled Release 30*:69-75, 1994; Yoshida et al., *J. Controlled Release 32*:97-102, 1994; Okano et al., *J. Controlled Release 36*:125-133, 1995; Chun and Kim, *J. Controlled Release 38*:39-47, 1996; D'Emanuele and Dinarvand, *Int'l J. Pharm. 118*:237-242, 1995; Katono et al., *J. Controlled Release 16*:215-228, 1991; Hoffman, "Thermally Reversible Hydrogels Containing Biologically Active Species," in Migliaresi et al. (eds.), *Polymers in Medicine III*, Elsevier Science Publishers B.V., Amsterdam, 1988, pp. 161-167; Hoffman, "Applications of Thermally Reversible Polymers and Hydrogels in Therapeutics and Diagnostics," in *Third International Symposium on Recent Advances in Drug Delivery Systems*, Salt Lake City, UT, Feb. 24-27, 1987, pp. 297-305; Gutowska et al.,

J. Controlled Release 22:95-104, 1992; Palasis and Gehrke, *J. Controlled Release 18*:1-12, 1992; Paavola et al., *Pharm. Res. 12*(12):1997-2002, 1995).

Representative examples of thermogelling polymers, and their gelatin temperature (LCST (°C)) include homopolymers such as poly(N-methyl-N-n-propylacrylamide), 19.8; poly(N-n-propylacrylamide), 21.5; poly(N-methyl-N-isopropylacrylamide), 22.3; poly(N-n-propylmethacrylamide), 28.0; poly(N-isopropylacrylamide), 30.9; poly(N, n-diethylacrylamide), 32.0; poly(N-isopropylmethacrylamide), 44.0; poly(N-cyclopropylacrylamide), 45.5; poly(N-ethylmethyacrylamide), 50.0; poly(N-methyl-N-ethylacrylamide), 56.0; poly(N-cyclopropylmethacrylamide), 59.0; poly(N-ethylacrylamide), 72.0. Moreover thermogelling polymers may be made by preparing copolymers between (among) monomers of the above, or by combining such homopolymers with other water soluble polymers such as acrylmonomers (*e.g.*, acrylic acid and derivatives thereof such as methylacrylic acid, acrylate and derivatives thereof such as butyl methacrylate, acrylamide, and N-n-butyl acrylamide).

Other representative examples of thermogelling polymers include cellulose ether derivatives such as hydroxypropyl cellulose, 41°C; methyl cellulose, 55°C; hydroxypropylmethyl cellulose, 66°C; and ethylhydroxyethyl cellulose, and Pluronics such as F-127, 10 - 15°C; L-122, 19°C; L-92, 26°C; L-81, 20°C; and L-61, 24°C.

A wide variety of forms may be fashioned by the polymeric carriers of the present invention, including for example, rod-shaped devices, pellets, slabs, or capsules (*see e.g.*, Goodell et al., *Am. J. Hosp. Pharm. 43*:1454-1461, 1986; Langer et al., "Controlled release of macromolecules from polymers", in *Biomedical Polymers, Polymeric Materials and Pharmaceuticals for Biomedical Use*, Goldberg, E.P., Nakagim, A. (eds.) Academic Press, pp. 113-137, 1980; Rhine et al., *J. Pharm. Sci. 69*:265-270, 1980; Brown et al., *J. Pharm. Sci. 72*:1181-1185, 1983; and Bawa et al., *J. Controlled Release 1*:259-267, 1985). Therapeutic agents may be linked by occlusion in the matrices of the polymer, bound by covalent linkages, or encapsulated in microcapsules. Within certain preferred embodiments of the invention, therapeutic compositions are provided in non-capsular formulations such as microspheres (ranging from nanometers to micrometers in size), pastes, threads of various size, films and sprays.

Preferably, therapeutic compositions of the present invention are fashioned in a manner appropriate to the intended use. Within certain aspects of the present invention, the therapeutic composition should be biocompatible, and release one or more therapeutic agents over a period of several days to months. For example, "quick release" or "burst" therapeutic compositions are provided that release greater than 10%, 20%, or 25% (w/v) of a therapeutic agent (*e.g.*, paclitaxel) over a period of 7 to 10 days. Such "quick release" compositions should, within certain embodiments, be capable of releasing chemotherapeutic levels (where applicable) of a desired agent. Within other embodiments, "low release" therapeutic compositions are provided that release less than 1% (w/v) of a therapeutic agent over a period of 7 to 10 days. Further, therapeutic compositions of the present invention should preferably be stable for several months and capable of being produced and maintained under sterile conditions.

Within certain aspects of the present invention, therapeutic compositions may be fashioned in any size ranging from 50 nm to 500 μm, depending upon the particular use. Alternatively, such compositions may also be readily applied as a "spray", which solidifies into a film or coating. Such sprays may be prepared from microspheres of a wide array of sizes, including for example, from 0.1 μm to 3 μm, from 10 μm to 30 μm, and from 30 μm to 100 μm.

Therapeutic compositions of the present invention may also be prepared in a variety of "paste" or gel forms. For example, within one embodiment of the invention, therapeutic compositions are provided which are liquid at one temperature (*e.g.*, temperature greater than 37°C, such as 40°C, 45°C, 50°C, 55°C or 60°C), and solid or semi-solid at another temperature (*e.g.*, ambient body temperature, or any temperature lower than 37°C). Such "thermopastes" may be readily made given the disclosure provided herein.

FORMULATION AND ADMINISTRATION

As noted above, anti-microtubule agents of the present invention may be formulated in a variety of forms suitable for administration. Further, the compositions of the present invention may be formulated to contain more than one anti-microtubule agents, to contain a variety of additional compounds, to have certain physical properties (*e.g.*, elasticity, a particular melting point, or a specified release rate). Within certain embodiments of the

invention, compositions may be combined in order to achieve a desired effect (*e.g.*, several preparations of microspheres may be combined in order to achieve both a quick and a slow or prolonged release of one or more anti-microtubule agents).

Anti-microtubule agents may be administered either alone, or in combination with pharmaceutically or physiologically acceptable carrier, excipients or diluents. Generally, such carriers should be nontoxic to recipients at the dosages and concentrations employed. Ordinarily, the preparation of such compositions entails combining the therapeutic agent with buffers, antioxidants such as ascorbic acid, low molecular weight (less than about 10 residues) polypeptides, proteins, amino acids, carbohydrates including glucose, sucrose or dextrins, chelating agents such as EDTA, glutathione and other stabilizers and excipients. Neutral buffered saline or saline mixed with nonspecific serum albumin are exemplary appropriate diluents.

CLINICAL ADMINISTRATION

The anti-microtubule agent or composition is administered into the pericardial sac (*i.e.*, the anatomical space between the two layers of the pericardium) by, for example, direct injection via specialized needle placed through the chest wall, through the myocardium, or through the vessel wall of the vasculature supplying the heart, with or without radiographic guidance. Similarly, the agent can be placed in the pericardial cavity under direct vision during open or endoscopic surgical procedures.

With direct injection, a catheter is placed in the pericardial sac preferably utilizing ultrasound, CT, fluoroscopic, MRI or endoscopic guidance (See *e.g.*, U.S. Patent Nos. 5,840,059 and 5,797,870). Within certain embodiments, specialized percutaneous injection devices are used to safely deliver the anti-microtubule agent into the pericardial sac via the thoracic wall (*e.g.*, with a Saphenous Vein Harvester such as GSI's ENDOsaph, or Comedicus Inc.'s PerDUCER (Pericardial Access Device)). In another embodiment, the antimicrotubule agent or composition is injected into the pericardium via a catheter (or other specialized injection device) placed trans-myocardially through the right or left ventricle. Similarly, the antimicrotubule agent or composition (*e.g.*, paclitaxel and a polymer) may be administered trans-myocardially through the right atrium. (See, *e.g.*, U.S. Patent Nos. 5,797,870 and 5,269,326). Briefly, the right atrium lies between the pericardium and the

epicardium. An appropriate catheter is guided into the right atrium and positioned parallel with the wall of the pericardium. This positioning allows piercing of the right atrium (either by the catheter, or by an instrument that is passed within the catheter), without risk of damage to either the pericardium or the epicardium. The catheter can then be passed into the pericardial space, or an instrument passed through the lumen of the catheter into the pericardial space. The anti-microtubule agent is then administered via the catheter into the pericardial space.

In open procedures, access to the pericardium, heart, or coronary vasculature is gained operatively, by, for example, sub-xiphoid entry, a thoracotomy, open heart surgery, or endoscopic procedures. Preferably, the thoracotomy should be minimal, through an intercostal space for example. Fluoroscopy, or ultrasonic visualization may be utilized to assist in any of these procedures. The anti-microtubule agent is then administered directly to the required site on the heart surface (*e.g.*, the coronary arteries) as a paste, gel, wrap or solution. This placement may be accomplished by the surgeon directly (open surgery) or via a delivery port in a endoscopic device (endoscopic surgery).

The anti-microtubule agent is administered in a dosage and formulation which results in clinical improvement of the patient. As mentioned previously, the compositions and methods described herein are suitable for the treatment of a variety of diseases of the heart and surrounding tissues, including, but not limited to: coronary stenosis, restenosis, in-stent restenosis, and atherosclerosis; surgical adhesions following cardiac surgery (open, endoscopic, or catheter –based); stenosis or failure of valve replacement surgery; restenosis following cardiac shunt procedures; accelerated atherosclerosis following cardiac transplantation; immunological rejection following cardiac transplantation (host vs. graft disease); cardiac rhythm abnormalities; rheumatic or inflammatory diseases with cardiac manifestations (*i.e.*, rheumatoid arthritis, systemic lupus erythematosis, vasculitis); infections of the myocardium or surrounding tissues; and/or primary or metastatic malignancy of the myocardium, pericardium (*e.g.*, malignant pericardial effussion), or surrounding tissues.

For the embodiments described below, an antimicrotubule agent such as paclitaxel is administered into the pericardium at a dosage ranging from 100 ug to 50 mg, depending on the mode of administration and the type of carrier, if any, for delivery. It

should be apparent to one of skill in the art that any method of gaining access to the pericardial sac would be acceptable for the purposes of this invention. It will also be apparent to one of skill in the art that other anti-microtubule agents would be acceptable for the purposes of this invention if given at biologically equivalent doses to those described for paclitaxel.

TREATMENT OF STENOSIS, RESTENOSIS, IN-STENT RESTENOSIS

For treatment of coronary stenosis, restenosis, or in-stent restenosis, a single direct injection of an anti-microtubule into the pericardial sac is the preferred intervention. This injection is administered before, during or after a vascular intervention such as balloon angioplasty (PTCA), stenting, atherectomy, laser ablation, rotary ablation, or surgical bypass (including open and endoscopic procedures). In a preferred embodiment, the anti-microtubule agent is administered as an aqueous solution or in a sustained-release form (*i.e.*, in association with a polymeric or nonpolymeric carrier) into the pericardial sac using the methods described previously. In a particularly preferred embodiment, paclitaxel (or an analogue or derivative thereof) is administered as a solution (*e.g.*, micellar paclitaxel) or in a sustained-release preparation (*e.g.*, PLA microspheres or hyaluronic acid microspheres containing paclitaxel) at a dose of 1 to 50 mg/m^2 which disperses throughout the pericardial space. The sustained-release preparation is designed to release therapeutic levels of paclitaxel over a period of several days to several months, depending on the indication.

For certain clinical indications (*e.g.*, the treatment of instent restenosis, restenosis following cardiac bypass surgery), the anti-microtubule agent may also be administered directly to the surface of the stented (or bypassed) coronary artery (or saphenous vein graft) to prevent closure of the stented vessel. If the drug is administered under direct vision (open surgical or endoscopic procedures), then an anti-microtubule agent contained in a paste or gel formulation (*e.g.*, paclitaxel contained in hyaluronic acid, in fibrin, or in poly-lactic acid) is the preferred embodiment. Here the physician is able to directly apply the anti-microtubule agent directly to the outer (adventitial) surface of the diseased coronary artery or tissue via the pericardial sac. Paclitaxel at a total dose of 1 to 75 mg/m^2 delivered over a period of 24 hours to 6 months in an injectable gel or paste is a particularly effective embodiment. Alternatively the anti-microtubule agent can be injected into the wall of the

coronary artery via needles/catheters suitable for this purpose. Although a single injection is preferred, when required, repeated injections can be performed to deliver the anti-microtubule agent to the pericardial space over longer periods of time.

Regardless of the methods employed, the anti-microtubule agent should be capable of reducing the signs and/or symptoms of coronary artery obstruction such as chest pain (angina), syncope, dyspnea, orthopnea, radiating pain (shoulder, arm pain), nausea, or diaphoresis. The treatment may also preserve luminal area following vascular intervention, reduce the incidence of total occlusion (myocardial infarction), prolong the effectiveness of PTCA or stenting, or preserve myocardial perfusion as demonstrated by standard cardiac function and radiographic methods.

TREATMENT OF ATHEROSCLEROSIS

For the treatment of progressive atherosclerosis, an anti-microtubule agent is injected periodically (*e.g.*, once every few months) directly into the pericardial sac utilizing the methods and devices described above . In a preferred embodiment, paclitaxel is administered as an aqueous solution or in a sustained-release form (*i.e.*, in association with a polymeric or nonpolymeric carrier). In a particularly preferred embodiment, paclitaxel (or an analogue or derivative thereof) is administered in a sustained-release preparation (*e.g.*, PLA microspheres or hyaluronic acid microspheres containing paclitaxel) at a dose of 1 to 50 mg/m^2 which disperses throughout the pericardial space. The sustained-release preparation is designed to release therapeutic levels of paclitaxel over a period of several days to several months. This dose is then repeated 1 to 4 times per year (or as required) to slow the progression of atherosclerosis or stabilize atherosclerotic plaques.

In the case of acelerated atherosclerosis associated with cardiac transplantation, paclitaxel can be administered in a slow release form that delivers a total dose of 1 to 75 mg/m^2 (preferably 10 to 50 mg/m^2) of drug over a selected period of time. The drug may be administered initially as a paste or gel placed at the time of transplant surgery. Subsequent drug administration is then achieved in the manner described in the previous paragraph. A preferred carrier for the drug is hyaluronic acid as this agent, in combination with paclitaxel, may help reduce the incidence of post-surgical pericardial adhesions.

Regardless of the methods employed, the anti-microtubule agent should be capable of reducing the signs and/or symptoms of coronary artery obstruction such as chest pain (angina), syncope, dyspnea, orthopnea, radiating pain (shoulder, arm pain), nausea, or diaphoresis. The treatment may also preserve coronary artery luminal diameter/area, reduce the incidence of total occlusion (myocardial infarction), or preserve myocardial perfusion as demonstrated by standard cardiac function and radiographic methods.

TREATMENT OF TRANSPLANT REJECTION

In the case of organ rejection associated with cardiac transplantation, paclitaxel can be administered in a slow release form that delivers a total dose of 1 to 75 mg/m^2 (preferably 10 to 50 mg/m^2) of drug over a selected period of time. The drug may be administered initially as a paste or gel placed at the time of transplant surgery. Subsequent drug administration is then achieved via direct pericardial injection.

The anti-microtubule agent is injected periodically (*e.g.*, once every few months) directly into the pericardial sac utilizing the methods and devices described previously. In a preferred embodiment, paclitaxel (or an analogue or derivative thereof) is administered in a sustained-release preparation (*e.g.*, PLA microspheres or hyaluronic acid microspheres containing paclitaxel) at a dose of 1 to 50 mg/m^2 which disperses throughout the pericardial space. The sustained-release preparation is designed to release therapeutic levels of paclitaxel over a period of several days to several months. A preferred carrier for the drug is hyaluronic acid as this agent, in combination with paclitaxel, may also help reduce the incidence of post-surgical pericardial adhesions. This dose is then repeated 1 to 4 times per year (or as required) to help prevent transplant rejection.

TREATMENT OF ARTERITIS AND OTHER RHEUMATIC CONDITIONS AFFECTING THE HEART

Several rheumatic diseases are associated with cardiac manifestations, such as arteritis, systemic lupus erythematosis, and rheumatoid arthritis. As described herein, anti-microtubule agents such as paclitaxel can be utilized in the systemic treatment of rheumatic diseases. Intrapericardial administration may be particularly effective in the management of pericarditis, coronary arteritis (*e.g.*, Kowasaki's disease, polyarteritis) and myocarditis

associated with these conditions. Local administration of an anti-microtubule agent into the pericardial sac can result in increased drug levels at the site of the disease while decreasing systemic exposure to the agent.

Briefly, the anti-microtubule agent is injected periodically (*e.g.*, once every few months) directly into the pericardial sac utilizing the methods and devices described previously. In a preferred embodiment, paclitaxel (or an analogue or derivative thereof) is administered in a sustained-release preparation (*e.g.*, PLA microspheres or hyaluronic acid microspheres containing paclitaxel) at a dose of 1 to 50 mg/m^2 which disperses throughout the pericardial space. The sustained-release preparation is designed to release therapeutic levels of paclitaxel over a period of several days to several months. A preferred carrier for the drug is hyaluronic acid as this agent, in combination with paclitaxel, may also help reduce the formation of pericardial adhesions. This dose is then repeated 1 to 4 times per year (or as required) to help prevent transplant rejection.

It should be noted that certain infections leading to pericarditis and/or myocarditis could also benefit from anti-microtubule therapy. In particular, certain paracytic infections (*e.g.*, malaria) are responsive to anti-microtubule drugs such as paclitaxel.

TREATMENT VALVULAR STENOSIS AND SHUNT RESTENOSIS

Cardiac surgery is often performed to replace the patient's leaking or obstructed valves with porcine or mechanical heart valves. A complication of this procedure is that scarring of the annular ring of the valve can lead to narrowing or stenosis of the valve and loss of function. In a similar manner, shunts are artificial connections created between chambers of the heart that allow blood to pass between chambers to reduce pressure in one of the chambers. These openings can also scar shut (or stenose) and limit the effectiveness of the procedure. As described herein, anti-microtubule agents, such as paclitaxel can be utilized in the prevention of scar tissue formation.

In a preferred embodiment, paclitaxel can be administered in a slow release form that delivers a total dose of 1 to 75 mg/m^2 (preferably 10 to 50 mg/m^2) of drug over a selected period of time. The drug may be administered initially as a paste or gel placed at the

time of valvular or shunt surgery. Subsequent drug administration is then achieved via direct pericardial injection.

The anti-microtubule agent is then injected periodically (*e.g.*, once every few months) directly into the pericardial sac utilizing the methods and devices described previously. In a preferred embodiment, paclitaxel (or an analogue or derivative thereof) is administered in a sustained-release preparation (*e.g.*, PLA microspheres or hyaluronic acid microspheres containing paclitaxel) at a dose of 1 to 50 mg/m^2 which disperses throughout the pericardial space. The sustained-release preparation is designed to release therapeutic levels of paclitaxel over a period of several days to several months. A preferred carrier for the drug is hyaluronic acid as this agent, in combination with paclitaxel, may also help reduce the incidence of post-surgical pericardial adhesions. This dose is then repeated 1 to 4 times per year (or as required) to help prevent stenosis of the valve or shunt.

The anti-microtubule agent should be capable of reducing the signs and/or symptoms of valvular obstruction or shunt obstruction such as chest pain (angina), syncope, dyspnea, orthopnea, paroxysmal nocturnal dyspnea, radiating pain (shoulder, arm pain), nausea, congestive heart failure, pulmonary edema, or hepatosplenomegaly.

TREATMENT OF CARDIAC ADHESIONS FOLLOWING SURGERY

Cardiac surgery for valve replacement or coronary artery bypass grafting (CABG) is an extremely common surgical procedure. A complication of this procedure is that scarring of the pericardium can lead to the formation of adhesions (+/- effusions) that impact negatively on cardiac contractility and function. As described in the following examples, anti-microtubule agents such as paclitaxel can be utilized in the prevention of surgical adhesion formation.

In a preferred embodiment, paclitaxel can be administered intraoperatively in a slow release paste or gel that delivers a total dose of 1 to 75 mg/m^2 (preferably 10 to 50 mg/m^2) of drug over a selected period of time. Subsequent drug administration is then achieved via direct pericardial injection.

The anti-microtubule agent is then injected periodically (*e.g.*, once every few months) directly into the pericardial sac utilizing the methods and devices described

previously. In a preferred embodiment, paclitaxel (or an analogue or derivative thereof) is administered in a sustained-release preparation (*e.g.*, PLA microspheres or hyaluronic acid microspheres containing paclitaxel) at a dose of 1 to 50 mg/m^2 which disperses throughout the pericardial space. The sustained-release preparation is designed to release therapeutic levels of paclitaxel over a period of several days to several months. A preferred carrier for the drug is hyaluronic acid as this agent, in combination with paclitaxel, may also help reduce the incidence of post-surgical pericardial adhesions. This dose is then repeated 1 to 4 times per year (or as required) to help prevent stenosis of the valve or shunt.

The anti-microtubule agent should be capable of reducing the signs and/or symptoms of pericardial adhesions such as chest pain, syncope, dyspnea, congestive heart failure, pericardial friction rubs, and decreased cardiac output.

TREATMENT OF MALIGNANT PERICARDIAL EFFUSIONS

Primary or metastatic malignancy can affect the heart leading to malignant pericarditis and pericardial effusions. Given that anti-microtubule agents are potent chemotherapeutic drugs, pericardial administration of these agents can be useful for palliative relief of this condition.

The anti-microtubule agent is injected periodically (*e.g.*, once every few days to months) directly into the pericardial sac utilizing the methods and devices described previously. In a preferred embodiment, paclitaxel (or an analogue or derivative thereof) is administered in a sustained-release preparation (*e.g.*, PLA microspheres or hyaluronic acid microspheres containing paclitaxel) at a dose of 50 to 350 mg/m^2 which disperses throughout the pericardial space. The sustained-release preparation is designed to release therapeutic chemotherapeutic levels of paclitaxel over a period of several days to several months. This dose is then repeated as required to help provide palliative relief to the patient.

TREATMENT OF CARDIAC RHYTHM DISORDERS

Numerous cardiac rhythm disorders have been described that lead to conditions ranging from mild discomfort to sudden cardiac death. Certain rhythm

abnormalities (*e.g.*, ablation of abnormal conduction pathways) may benefit from the local application of cytotoxic anti-microtubule therapy.

As should be readily evident, with any of the embodiments discussed herein, the anti-microtubule agent (*e.g.*, paclitaxel) may be administered along with other therapeutics.

The following examples are offered by way of illustration, and not by way of limitation.

EXAMPLES

EXAMPLES

EXAMPLE 1

MANUFACTURE OF AND USE OF DIBLOCK CO-POLYMERS FOR FORMULATING THE DELIVERY OF ANTI-MICROTUBULE AGENTS.

One formulation for paclitaxel is comprised of amphiphilic diblock copolymers which in aqueous solutions form micelles consisting of a hydrophobic core and a hydrophilic shell in water. Briefly, diblock copolymers of poly(DL-lactide)-block-methoxy polyethylene glycol (PDLLA-MePEG), polycaprolactone-block methoxy polyethylene glycol (PCL-MePEG) and poly(DL-lactide-co-caprolactone)-block-methoxy polyethylene glycol (PDLLACL-MePEG) can be synthesized using a bulk melt polymerization procedure, or similar methods. Briefly, given amounts of monomers DL-lactide, caprolactone and methoxy polyethylene glycols with different molecular weights were heated (130°C) to melt under the bubbling of nitrogen and stirred. The catalyst stannous octoate (0.2% w/w) was added to the molten monomers. The polymerization was carried out for 4 hours. The molecular weights, critical micelle concentrations and the maximum paclitaxel loadings were measured with GPC, fluorescence, and solubilization testing, respectively. High paclitaxel carrying capacities were obtained. The ability of solubilizing paclitaxel depends on the compositions and concentrations of the copolymers. PDLLA-MePEG gave the most stable solubilized paclitaxel.

The strong association within the internal core of the polymeric micelles presents a high capacity environment for carrying hydrophobic drugs such as paclitaxel. The drugs can be covalently coupled to block copolymers to form a micellar structure or can be physically incorporated within the hydrophobic cores of the micelles. The mechanisms of drug release from the micelles include diffusion from the core and the exchange between the single polymer chains and the micelles. The small size of the micelles (normally less than 100 nm) will eliminate the difficulties associated with injecting larger particles.

EXAMPLE 2

MODIFICATION OF PACLITAXEL RELEASE FROM THERMOPASTE USING PDLLA-PEG-PDLLA AND LOW MOLECULAR WEIGHT POLY(D,L, LACTIC ACID)

A. Preparation of PDLLA-PEG-PDLLA and low molecular weight PDLLA

DL-lactide was purchased from Aldrich. Polyethylene glycol (PEG) with molecular weight 8,000, stannous octoate, and DL-lactic acid were obtained from Sigma. Poly-ε-caprolactone (PCL) with molecular weight 20,000 was obtained from Birmingham Polymers (Birmingham, AL). Paclitaxel was purchased from Hauser Chemicals (Boulder, CO). Polystyrene standards with narrow molecular weight distributions were purchased from Polysciences (Warrington, PA). Acetonitrile and methylene chloride were HPLC grade (Fisher Scientific).

The triblock copolymer of PDLLA-PEG-PDLLA was synthesized by a ring opening polymerization. Monomers of DL-lactide and PEG in different ratios were mixed and 0.5 wt% stannous octoate was added. The polymerization was carried out at 150°C for 3.5 hours. Low molecular weight PDLLA was synthesized through polycondensation of DL-lactic acid. The reaction was performed in a glass flask under the conditions of gentle nitrogen purge, mechanical stirring, and heating at 180°C for 1.5 hours. The PDLLA molecular weight was about 800 measured by titrating the carboxylic acid end groups.

B. Manufacture of paste formulations

Paclitaxel at loadings of 20% or 30% was thoroughly mixed into either the PDLLA-PEG-PDLLA copolymers or blends of PDLLA:PCL 90:10, 80:20 and 70:30 melted

at about 60°C. The paclitaxel-loaded pastes were weighed into 1 ml syringes and stored at 4°C.

C. *In vitro* release of paclitaxel

A small pellet of 20% paclitaxel-loaded PDLLA:PCL paste (about 2 mg) or a cylinder (made by extruding molten paste through a syringe) of 20% paclitaxel-loaded PDLLA-PEG-PDLLA paste were placed into capped 14 ml glass tubes containing 10 ml phosphate buffered saline (pH 7.4) with 0.4 g/L albumin. The tube was incubated at 37°C with gentle rotational mixing. The supernatant was withdrawn periodically for paclitaxel analysis and replaced with fresh PBS/albumin buffer. The supernatant (10 ml) was extracted with 1 ml methylene chloride. The water phase was decanted and the methylene chloride phase was dried under a stream of nitrogen at 60°C. The dried residue was reconstituted in a 40:60 water:acetonitrile mixture and centrifuged at 10,000g for about 1 min. The amount of paclitaxel in the supernatant was then analyzed by HPLC. HPLC analysis was performed using a 110A pump and C-8 ultrasphere column (Beckman), and a SPD-6A UV detector set at 232 nm, a SIL-9A autoinjector and a C-R3A integrator (Shimadzu). The injection volume was 20 µl and the flow rate was 1 ml/minute. The mobile phase was 58% acetonitrile, 5% methanol, and 37% distilled water.

D. Results and Discussion

The molecular weight and molecular weight distribution of PDLLA-PEG-PDLLA, relative to polystyrene standards, were measured by GPC. The intrinsic viscosity of the copolymer in $CHCl_3$ at 25°C was determined using a Canon-Fenske viscometer. The molecular weight and intrinsic viscosity decreased with increasing PEG content. The polydispersities of PDLLA-PEG-PDLLA with PEG contents of 10% - 40% were from 2.4 to 3.5. However, the copolymer with 70% PEG had a narrow molecular weight distribution with a polydispersity of 1.21. This might be due to a high PEG content reducing the chance of side reactions such as transesterfication which results in a wide distribution of polymer molecular weights. Alternatively, a coiled structure of the hydrophobic-hydrophilic block copolymers may result in an artificial low polydispersity value.

The PEG and PDLLA-PEG-PDLLA with PEG contents of 70% and 40% showed endothermic peaks with decreasing enthalpy and temperature as the PEG content of the copolymer decreased. The endothermic peaks in the copolymers of 40% and 70% PEG were probably due to the melting of the PEG region, indicating the occurrence of phase separation. While pure PEG had a sharp melting peak, the copolymers of both 70% and 40% PEG showed broad peaks with a distinct shoulder in the case of 70% PEG. The broad melting peaks may have resulted from the interference of PDLLA with the crystallization of PEG. The shoulder in the case of 70% PEG might represent the glass transition of the PDLLA region. No thermal changes occurred in the copolymers with PEG contents of 10%, 20% and 30% in a temperature range of 10 - 250°C, indicating that no significant crystallization (therefore may be the phase separation) had occurred.

DSC thermograms of PDLLA:PCL (70:30, 80:20, 90:10) blends without paclitaxel or with 20% paclitaxel showed an endothermic peak at about 60°C, resulting from the melting of PCL. Due to the amorphous nature of the PDLLA and its low molecular weight (800), melting and glass transitions of PDLLA were not observed. No thermal changes due to the recrystallization or melting of paclitaxel was observed.

PDLLA-PEG-PDLLA copolymers of 20% and 30% PEG content were selected as optimum formulation materials for the paste for the following reasons: PDLLA-PEG-PDLLA of 10% PEG could not be melted at a temperature of about 60°C; the copolymers of 40% and 70% PEG were readily melted at 60°C, and the 20% and 30% PEG copolymer became a viscous liquid between 50°C to 60°C; and the swelling of 40% and 70% PEG copolymers in water was very high resulting in rapid dispersion of the pastes in water.

EXAMPLE 3

PROCEDURE FOR PRODUCING NANOPASTE

Nanopaste is a suspension of microspheres in a hydrophilic gel. Within one aspect of the invention, the gel or paste can be smeared over tissue as a method of locating drug-loaded microspheres close to the target tissue. Being water based, the paste soon becomes diluted with bodily fluids causing a decrease in the stickiness of the paste and a

tendency of the microspheres to be deposited on nearby tissue. A pool of microsphere encapsulated drug is thereby located close to the target tissue.

Reagents and equipment which were utilized within these experiments include glass beakers, Carbopol 925 (pharmaceutical grade, Goodyear Chemical Co.), distilled water, sodium hydroxide (1 M) in water solution, sodium hydroxide solution (5 M) in water solution, microspheres in the 0.1 lm to 3 lm size range suspended in water at 20% w/v (see previous).

1. PREPARATION OF 5% W/V CARBOPOL GEL

A sufficient amount of carbopol was added to 1 M sodium hydroxide to achieve a 5% w/v solution. To dissolve the carbopol in the 1 M sodium hydroxide, the mixture was allowed to sit for approximately one hour. During this time period, the mixture was stirred and, after one hour, the pH was adjusted to 7.4 using 5 M sodium hydroxide until the carbopol was fully dissolved. Once a pH of 7.4 was achieved, the gel was covered and allowed to sit for 2 to 3 hours.

2. PROCEDURE FOR PRODUCING NANOPASTE

A sufficient amount of 0.1 μm to 3 μm microspheres was added to water to produce a 20% suspension of the microspheres. Carbopol gel (8 ml of the 5% w/v) was placed into a glass beaker and 2 ml of the 20% microsphere suspension was added. The mixture was stirred to thoroughly disperse the microspheres throughout the gel. This mixture was stored at 4°C.

EXAMPLE 4

POLYMERIC COMPOSITIONS WITH INCREASED CONCENTRATIONS OF PACLITAXEL

PDLLA-MePEG and PDLLA-PEG-PDLLA are block copolymers with hydrophobic (PDLLA) and hydrophilic (PEG or MePEG) regions. At appropriate molecular weights and chemical composition, they may form tiny aggregates of hydrophobic PDLLA

core and hydrophilic MePEG shell. Paclitaxel can be loaded into the hydrophobic core, thereby providing paclitaxel with an increased "solubility."

A. Materials

D,L-lactide was purchased from Aldrich, Stannous octoate, poly (ethylene glycol) (mol. wt. 8,000), MePEG (mol. wt. 2,000 and 5,000) were from Sigma. MePEG (mol. wt. 750) was from Union Carbide. The copolymers were synthesized by a ring opening polymerization procedure using stannous octoate as a catalyst (Deng et al., *J. Polym. Sci., Polym, Lett. 28*:411-416, 1990; Cohn et al., *J. Biomed, Mater. Res. 22*: 993-1009, 1988).

For synthesizing PDLLA-MePEG, a mixture of DL-lactide/MePEG/stannous octoate was added to a 10 milliliter glass ampoule. The ampoule was connected to a vacuum and sealed with flame. Polymerization was accomplished by incubating the ampoule in a 150 °C oil bath for 3 hours. For synthesizing PDLLA-PEG-PDLLA, a mixture of D,L-lactide/PEG/stannous octoate was transferred into a glass flask, sealed with a rubber stopper, and heated for 3 hours in a 150°C oven. The starting compositions of the copolymers are given in Tables 1 and 2. In all the cases, the amount of stannous octoate was 0.5% - 0.7%.

B. Methods

The polymers were dissolved in acetonitrile and centrifuged at 10,000 g for 5 minutes to discard any non-dissolvable impurities. Paclitaxel acetonitrile solution was then added to each polymer solution to give a solution with paclitaxel (paclitaxel + polymer) of 10% wt. The solvent acetonitrile was then removed to obtain a clear paclitaxel/PDLLA-MePEG matrix, under a stream of nitrogen and 60°C warming. Distilled water, 0.9% NaCl saline, or 5% dextrose was added at four times weight of the matrix. The matrix was finally "dissolved" with the help of vortex mixing and periodic warming at 60°C. Clear solutions were obtained in all the cases. The particle sizes were all below 50 nm as determined by a submicron particle sizer (NICOMP Model 270). The formulations are given in Table 1.

Table 1. Formulations of Paclitaxel/PDLLA-MePEG*

PDLLA-MePEG	Dissolving Media	Paclitaxel Loading (final paclitaxel concentrate)
2000/50/50	water	10% (20 mg/ml)
2000/40/60	water	10% (20 mg/ml)
2000/50/50	0.9% saline	5% (10 mg/ml)
2000/50/50	0.9% saline	10% (20 mg/ml)
2000/50/50	5% dextrose	10% (10 mg/ml)
2000/50/50	5% dextrose	10% (20 mg/ml)

In the case of PDLLA-PEG-PDLLA (Table 2), since the copolymers cannot dissolve in water, paclitaxel and the polymer were co-dissolved in acetone. Water or a mixture of water/acetone was gradually added to this paclitaxel polymer solution to induce the formation of paclitaxel/polymer spheres.

Table 2. Composition of PDLLA-PEG-PDLLA

Copolymer Name	Wt. of PEG (g)	Wt. of DL-lactide (g)
PDLLA-PEG-PDLLA 90/10	1	9
PDLLA-PEG-PDLLA 80/20	2	8
PDLLA-PEG-PDLLA 70/30	3	7
PDLLA-PEG-PDLLA 60/40	4	6
PDLLA-PEG-PDLLA 30-/70	14	6

* PEG molecular weight. 8,000.

C. Results

Many of the PDLLA-MePEG compositions form clear solutions in water, 0.9% saline, or 5% dextrose, indicating the formation of tiny aggregates in the range of nanometers. Paclitaxel was loaded into PDLLA-MePEG micelles successfully. For example, at % loading (this represents 10 mg paclitaxel in 1 ml paclitaxel/PDLLA-MePEG/aqueous

system), a clear solution was obtained from 2000-50/50 and 2000-40/60. The particle size was about 60 nm.

EXAMPLE 5

MANUFACTURE OF MICELLAR PACLITAXEL

Poly(DL-lactide)-block-methoxypolyethylene glycol (PDLLA-block-MePEG) with a MePEG molecular weight of 2000 and a PDLLA:MePEG weight ratio 40:60 is used as the micellar carrier for the solubilization of paclitaxel. PDLLA-MePEG 2000-40/60 (polymer) is an amphiphilic diblock copolymer that dissolves in aqueous solutions to form micelles with a hydrophobic PDLLA core and hydrophilic MePEG shell. Paclitaxel is physically trapped in the hydrophobic PDLLA core to achieve the solubilization.

The polymer was synthesized from the monomers methoxypolyethylene glycol and DL-lactide in the presence of 0.5% w/w stannous octoate through a ring opening polymerization. Stannous octoate acted as a catalyst and participated in the initiation of the polymerization reaction. Stannous octoate forms a number of catalytically reactive species which complex with the hydroxyl group of MePEG and provide an initiation site for the polymerization. The complex attacks the DL-lactide rings and the rings open up and are added to the chain, one-by-one, forming the polymer. The calculated molecular weight of the polymer is 3,333.

All reaction glassware was washed and rinsed with Sterile Water for Irrigation, USP, dried at 37°C, followed by depyrogenation at 250°C for at least 1 hour. MePEG (240 g) and DL-lactide (160 g) were weighed and transferred to a round bottom glass flask using a stainless steel funnel. A 2 inch Teflon coated magnetic stir bar was added to the flask. The flask was sealed with a glass stopper and then immersed to the neck in a 140°C oil bath. After the MePEG and DL-lactide melted, 2 ml of 95% stannous octoate (catalyst) was added to the flask. The flask was vigorously shaken immediately after the addition to ensure rapid mixing and then returned to the oil bath. The reaction was allowed to proceed for an additional 6 hours with heat and stirring. The liquid polymer was then poured into a stainless steel tray, covered and left in a chemical fume hood overnight (about 16 hours). The polymer solidified in the tray. The top of the tray was sealed using Parafilm®. The sealed tray

containing the polymer was placed in a freezer at -20 ± 5°C for at least 0.5 hour. The polymer was then removed from the freezer, broken up into pieces and transferred to glass storage bottles and stored refrigerated at 2 to 8°C.

Preparation of a 50 mg/m^2 Dose

Preparation of the bulk and filling of paclitaxel/polymer matrix was accomplished essentially as follows. Reaction glassware was washed and rinsed with Sterile Water for Irrigation USP, and dried at 37°C, followed by depyrogenation at 250°C for at least 1 hour. First, a phosphate buffer (0.08 M, pH 7.6) was prepared. The buffer was dispensed at the volume of 10 ml per vial. The vials were heated for 2 hours at 90°C to dry the buffer. The temperature was then raised to 160°C and the vials dried for an additional 3 hours.

The polymer was dissolved in acetonitrile at 15% w/v concentration with stirring and heat. The polymer solution was then centrifuged at 3000 rpm for 30 minutes. The supernatant was poured off and set aside. Additional acetonitrile was added to the precipitate and centrifuged a second time at 3000 rpm for 30 minutes. The second supernatant was pooled with the first supernatant. Paclitaxel was weighed and then added to the supernatant pool. The solution was brought to the final desired volume with acetonitrile.

The paclitaxel/polymer matrix solution is dispensed into the vials containing previously dried phosphate buffer at a volume of 10 ml per vial. The vials are then vacuum dried to remove the acetonitrile. The paclitaxel/polymer matrix is then terminally sterilized by irradiation with at least 2.5 Mrad Cobalt-60 (Co-60) x-rays.

EXAMPLE 6

MANUFACTURE OF MICROSPHERES

The equipment used for the manufacture of microspheres include: 200 ml water jacketed beaker (Kimax or Pyrex), Haake circulating water bath, overhead stirrer and controller with 2 inch diameter (4 blade, propeller type stainless steel stirrer - Fisher brand), 500 ml glass beaker, hot plate/stirrer (Corning brand), 4 X 50 ml polypropylene centrifuge tubes (Nalgene), glass scintillation vials with plastic insert caps, table top centrifuge (GPR Beckman), high speed centrifuge- floor model (JS 21 Beckman), Mettler analytical balance

(AJ 100, 0.1 mg), Mettler digital top loading balance (AE 163, 0.01 mg), automatic pipetter (Gilson). Reagents include PCL (mol. wt. 10,000 to 20,000; Polysciences, Warrington Pennsylvania, USA), "washed" (see later method of "washing") EVA, PLA (mol. wt. 15,000 to 25,000; Polysciences), polyvinyl alcohol ("PVA" - mol. wt. 124,000 to 186,000; 99% hydrolyzed; Aldrich Chemical Co., Milwaukee, Wisconsin, USA), DCM or "methylene chloride"; HPLC grade Fisher scientific, and distilled water.

A. Preparation of 5% (w/v) Polymer Solutions

PCL (1.00 g) or PLA, or 0.50 g each of PLA and washed EVA was weighed directly into a 20 ml glass scintillation vial. Twenty milliliters of DCM was then added. The vial was capped and stored at room temperature (25°C) for one hour (occasional shaking may be used), or until all the polymer was dissolved. The solution may be stored at room temperature for at least two weeks.

B. Preparation of 5% (w/v) Stock Solution of PVA

Twenty-five grams of PVA was weighed directly into a 600 ml glass beaker and 500 ml of distilled water was added, along with a 3 inch Teflon coated stir bar. The beaker was covered with glass to decrease evaporation losses, and placed into a 2000 ml glass beaker containing 300 ml of water. The PVA was stirred at 300 rpm at 85°C (Corning hot plate/stirrer) for 2 hours or until fully dissolved. Dissolution of the PVA was determined by a visual check; the solution should be clear. The solution was then transferred to a glass screw top storage container and stored at 4°C for a maximum of two months. The solution, however must be warmed to room temperature before use or dilution.

C. Procedure for Producing Microspheres

Based on the size of microspheres being made (see Table 1), 100 ml of the PVA solution (concentrations given in Table 1) was placed into the 200 ml water jacketed beaker. Haake circulating water bath was connected to this beaker and the contents were allowed to equilibrate at 27°C (+/-1°C) for 10 minutes. Based on the size of microspheres being made (see Table I), the start speed of the overhead stirrer was set, and the blade of the overhead stirrer placed half way down in the PVA solution. The stirrer was then started, and

10 ml of polymer solution (polymer solution used based on type of microspheres being produced) was then dripped into the stirring PVA over a period of 2 minutes using a 5 ml automatic pipetter. After 3 minutes the stir speed was adjusted (see Table 1), and the solution stirred for an additional 2.5 hours. The stirring blade was then removed from the microsphere preparation, and rinsed with 10 ml of distilled water so that the rinse solution drained into the microsphere preparation. The microsphere preparation was then poured into a 500 ml beaker, and the jacketed water bath washed with 70 ml of distilled water, which was also allowed to drain into the microsphere preparation. The 180 ml microsphere preparation was then stirred with a glass rod, and equal amounts were poured into four polypropylene 50 ml centrifuge tubes. The tubes were then capped, and centrifuged for 10 minutes (force given in Table 1). Forty-five milliliters of the PVA solution was drawn off of each microsphere pellet.

TABLE 1

PVA concentrations, stir speeds, and centrifugal force requirements for each diameter range of microspheres.

PRODUCTION STAGE	MICROSPHERE DIAMETER RANGES		
	30 μm to 100 μm	10 μm to 30 μm	0.1 μm to 3 μm
PVA concentration	2.5% (w/v) (*i.e.*,) dilute 5% stock with distilled water	5% (w/v) (*i.e.*, undiluted stock)	3.5% (w/v) (*i.e.*, dilute 5% stock with distilled water
Starting Stir Speed	500 rpm +/- 50 rpm	500 rpm +/- 50 rpm	3000 rpm +/- 200 rpm
Adjusted Stir Speed	500 rpm +/- 50 rpm	500 rpm +/- 50 rpm	2500 rpm +/- 200 rpm
Centrifuge Force	1000 g +/- 100 g (Table top model)	1000 g +/- 100 g (Table top model)	10 000 g +/- 1000 g (High speed model)

Five milliliters of distilled water was then added to each centrifuge tube and vortexed to resuspend the microspheres. The four microsphere suspensions were then pooled into one centrifuge tube along with 20 ml of distilled water, and centrifuged for another 10 minutes (force given in Table 1). This process was repeated two additional times for a total of three washes. The microspheres were then centrifuged a final time, and resuspended in 10

ml of distilled water. After the final wash, the microsphere preparation was transferred into a preweighed glass scintillation vial. The vial was capped, and left overnight at room temperature (25°C) in order to allow the microspheres to sediment out under gravity. Since microspheres which fall in the size range of 0.1 um to 3 um do not sediment out under gravity, they were left in the 10 ml suspension.

D. Drying of 10 μm to 30 μm or 30 μm to 100 μm Diameter Microspheres

After the microspheres sat at room temperature overnight, the supernatant was drawn off of the sedimented microspheres. The microspheres were allowed to dry in the uncapped vial in a drawer for a period of one week or until they were fully dry (vial at constant weight). Faster drying may be accomplished by leaving the uncapped vial under a slow stream of nitrogen gas (flow approx. 10 ml/minute.) in the fume hood. When fully dry (vial at constant weight), the vial was weighed and capped. The labeled, capped vial was stored at room temperature in a drawer. Microspheres were normally stored no longer than 3 months.

E. Determining the Concentration of 0.1 μm to 3 μm Diameter Microsphere Suspension

This size range of microspheres did not sediment out, so they were left in suspension at 4°C for a maximum of four weeks. To determine the concentration of microspheres in the 10 ml suspension, a 200 μl sample of the suspension was pipetted into a 1.5 ml preweighed microfuge tube. The tube was then centrifuged at 10,000 g (Eppendorf table top microfuge), the supernatant removed, and the tube allowed to dry at 50°C overnight. The tube was then reweighed in order to determine the weight of dried microspheres within the tube.

F. Manufacture of Paclitaxel Loaded Microsphere

In order to prepare paclitaxel containing microspheres, an appropriate amount of weighed paclitaxel (based upon the percentage of paclitaxel to be encapsulated) was placed directly into a 20 ml glass scintillation vial. Ten milliliters of an appropriate polymer solution was then added to the vial containing the paclitaxel, which was then vortexed until the paclitaxel dissolved.

Microspheres containing paclitaxel may then be produced essentially as described above in steps (C) through (E).

EXAMPLE 7

MANUFACTURE OF PACLITAXEL-LOADED STAR-SHAPED POLY(LACTIC ACID) (PLA) AND POLY(LACTIDE-CO-GLYCOLIC ACID) (PLGA) (PEG) MICROSPHERES

Microspheres containing 5, 10 or 20% paclitaxel in low molecular weight star-shaped PLA and PLGA (M.W. ≈ 10,000 by Gel Permeation Chromatography) were prepared by an oil-in-water emulsification technique. Briefly, the appropriate weights of the paclitaxel and 0.5 polymer were dissolved in 10 ml of dichloromethane and emulsified with a overhead propeller stirrer at the level of 3 (Fisher Scientific) into 100 ml 1% polyvinyl alcohol solution for about 3 hours. The formed microspheres were sieved and dried under vacuum at a temperature below 10°C. Yield of microspheres in the desired size range (53 - 90 μm) was about 50% and the encapsulation efficiency of paclitaxel in microspheres was about 98%.

Release studies were done by placing 2.5 mg of said microspheres in a 15 ml Teflon capped tube (with 10 ml phosphate buffer saline with albumin). Sampling daily (three sampling at the first day) to maintain the sink condition. Release study data showed that paclitaxel was released from the star-shaped microspheres 3 to 10 times faster than the conventional linear PLA and PLGA microspheres.

EXAMPLE 8

INTRAPERICARDIAL MICELLAR PACLITAXEL ADMINISTRATION IN A PORCINE MODEL

Juvenile farm pigs of approximately 20kg weight receive angiography to permit arterial measurement. Balloon injury is then performed alternatively in the LAD or LC at an overstretch ratio of 1.3 to 1 (see Figure 1). The alternative vessel receives a stent injury at the same overstretch ratio. Pericardial access and installation is obtained utilizing PerDUCER devices by Comedicus, Inc. (Minneapolis, MN).

The swine each receive a single infusion of unloaded micelles (control), or, 10 mg or 50 mg paclitaxel loaded micelles. The formulation containing 10 mg of paclitaxel demonstrated a marked improvement as shown in Figures 2 through 6.

EXAMPLE 9

TESTING OF POLYMER BIOCOMPATIBILITY WHEN DELIVERED INTO THE PERICARDIAL CHAMBER OF RABBITS

The objective of this study was to examine the biocompatibility of a number of controlled drug release polymers for the treatment of blood vessel disease when released into the intrapericardial cavity (the cavity between the membrane surrounding the heart and the heart.

Briefly, rabbits were anesthesized and maintained on a respirator with halothane. Following standard surgical procedures to expose the chest cavity, the pericardial sac was identified and punctured with the needle and approximately 1 mL of the polymer in saline was injected. The layers of muscle and skin were then sutured and animals recovered. At the two-week timepoint, animals were euthanized, and the chest cavity opened. Tissues (pericardial membrane, and heart) were examined for adhesion formation and inflammation including erythema, fluid, necrosis, and thickening of the pericardial membrane. Tissues including the heart and pericardial membrane were prepared for histological analysis.

Three groups of rabbits were tested. These included saline (N=4), a hyaluronic acid formulation (N=4) and a Poly-lactic acid microsphere formulation (N=2). The four rabbits injected (1ml) with saline and the 4 animals injected (1ml) with hyaluronic acid paste (20 mg/ml and 40 mg/ml) did not show any sign of toxicity at necropsy. A small area (1x1cm) of white soft material was present on the left ventricle close to the site of injection in the animals injected with microspheres (4mg/ml). The pericardium did not adhere to this tissue. The amount of fat surrounding the heart was remarkable in all animals and prevented thorough inspection of the pericardium at necropsy. Histology of the pericardial tissue following application of these formulations was conducted and did not show evidence of a chronic inflammatory reaction from the polymers.

These results demonstrate that the hyaluronic acid formulation and PLA microspheres are suitable polymers for intrapericardial delivery of drugs.

EXAMPLE 10

SURGICAL ADHESIONS

PROTOCOL

The rabbit uterine horn model was conducted essentially as described by Wiseman *et al.*, 1992 (Journal of Reproductive Medicine, 37: 766-770), with hemostasis. New Zealand female white rabbits were anesthetized and a midline incision made through the skin and the abdominal wall. Both uterine horns were located and exteriorized. Using a French Catheter Scale, the diameter of each uterine horn was measured and recorded. Only those rabbits with uterine horns measuring size 8 to 16, inclusive, on the French scale were used. Using a number 10 scalpel blade, 5 cm lengths of each uterine horn, approximately 1 cm from the uterine bifurcation, were scraped, 40 times per side, until punctuate bleeding. Hemostasis was achieved by tamponade.

Animals were randomized to receive: no treatment (Surgical Control); polymer Vehicle Control; paclitaxel (0.1% in vehicle); and paclitaxel (1% in vehicle). Test agent (0.4 to 2.5 ml) was applied over the horns via an 18 gauge needle. Uterine horns were replaced into the pelvis and the abdominal incision closed.

At 18, 31, 32, 33 and 60 days after surgery, animals were euthanized by intravenous injection of sodium pentobarbital (120 mg/ml; 1 ml/kg). Body weights of the animals were recorded. The abdomen was opened and the surgical site inspected. Adhesions were graded by a blinded observer as follows:

Extent of Adhesions The total length (cm) of each uterine horn involved with adhesions was estimated and recorded.

Tenacity (Severity) of Adhesions Adhesions were grades as 0 (absent), 1.0 (filmy adhesions) and 2.0 (tenacious, requiring sharp dissection).

Degree of Uterine Convolution The degree of uterine convolution was recorded according to the following scale:

No convolution:	Straight lengths of adherent or non-adherent horns which are clearly discerned.
Party convoluted:	Horns have adhesions and 50%-75% of the horn length is entangled preventing discernment of straight portions.
Completely convoluted:	It is impossible to discern uterine anatomy because the horn is completely entangled.

RESULTS

All animals maintained or gained weight during the study period. By inspection, there appeared to be no differences in average weight gain between the groups.

By inspection the extent of adhesion formation did not appear to vary with the time, in each group. Thus data for each group have been pooled. Adhesions formed in surgical controls to an extent consistent with historical data for this model. Paclitaxel exhibited a dose-dependent reduction in the extent of adhesions from 4.781 ± 0.219 cm in the Vehicle Control Group (N=8) to 2.925 ± 0.338 cm ($p<0.05$) and 2.028 ± 0.374 cm ($p<0.01$) in the 0.1% (N=10) and 1% (N=9) paclitaxel groups, respectively (Table 1).

Table 1

Effect of Paclitaxel on Adhesion Formulation in a Rabbit Uterine Horn Model

Group	*Extent*[1]	*Adhesion-Free*[2]	*Convolution*[3]	*N*
B. Vehicle Control	4.781 (0.219)	0/16	3/6/7	8
D. 0.1% paclitaxel	2.925 (0.338)*	0/20	16/2/2†	10
A. 1% paclitaxel	2.028 (0.374)**	0/18	18/0/0‡	9
C. Surgical Control	2.700 (0.407)**	0/20	16/2/2†	10

[1] Length of uterine horn with adhesions, cm (± Standard Error of the Mean)

[2] Number of uterine horns free of adhesions/total

[3] Number of uterine horns with no convolution/partial convolution/full convolution

* $p < 0.05$ (Dunnett's test); $p < 0.01$ *Student's t test) vs Vehicle Control unequal variance

** $p < 0.01$ (Dunnett's test), vs Vehicle Control

† $p = 0.0031$ (Fisher's Exact Test), vs Vehicle Control, Convolution classed as Present/Absent
$x^2 = 8.251$

‡ $p = <0.0001$ (Fisher's Exact Test), vs Vehicle Control, Convolution classed as Present/Absent
$x^2 = 17.07$

The degree of uterine convolution was also reduced in the 0.1% paclitaxel ($p = 0.0031$) and 1% paclitaxel ($p<0.0001$) groups.

EXAMPLE 11

EFFECTS OF MICELLAR PACLITAXEL HYALURONIC GEL IN AN ANIMAL MODEL OF SURGICAL ADHESIONS

The use of micellar paclitaxel hyaluronic acid gel to reduce adhesion formation is examined in the rat cecal abrasion model of surgical adhesions. The formulation would be applicable for application through the intrapericardial method of delivery.

METHODS

The rat cecal abrasion model is a well-established model of surgical adhesions. Male Sprague Dawley rats weighing 300 – 400 g were anaesthetized and maintained on 1.5 – 2% Halothane. The abdomen was shaved and scrubbed with an alcohol-based antiseptic wash, draped and opened with a central laparotomy incision of 3 – 4 cm within a sterile field. The cecum and large bowel were externalized with sterile swabs, and the cecum supported by a sponge such that contents could be evacuated into the large bowel. Both caecal surfaces were then stroked 45 times with a #10 scalpel blade to produce erythema and punctated bleeding. In no case did this treatment yield sustained bleeding requiring ligation. Each stroke spanned most of the caecal diameter (approximately 1 cm), and extended along 1.5 cm of the caecal extremity. After a delivery of a total of 90 strokes, the integrity of the tissue was confirmed, and cecum and large bowel were replaced in the pelvis.

One of two doses of Micellar Paclitaxel, (0.6 mg or 1.2 mg total Paclitaxel, or Hyaluronic acid gel with Micelles alone, were contained in a 3 mL bolus. The formulation was directed by syringe into the abdominal quadrant surrounding the damaged cecum, and the laparotomy was closed in two layers. Animals were warmed and monitored until fully recovered from the anaesthetic, and subsequently housed separately with food and water *ad libitum*, for one week.

EVALUATION OF ADHESIONS

One week following surgery, animals were euthanized with Sodium Pentobarbital and the abdomen opened for examination. Adhesions over, and adjacent to the cecum were rated according to the following findings: 0: No adhesions; 1: Filmy or stranded adhesions connected the cecum with omentum or adjacent intestines; 2: Cohesive adhesions tethered the cecum, which required aggressive blunt dissection; 3: Sharp dissection of adhesions was required to free the cecum from surrounding tissues. Fractions were assigned where a variety of ratings seemed applicable.

RESULTS

The use of hyaluronic acid gel reduced the severity of surgical adhesions by approximately 15%. The addition of micellar paclitaxel to the hyaluronic acid gel resulted in a dose-dependent reduction in the mean severity of adhesions. In addition, the percent of rats

showing an adhesion score greater than 2.5 was reduced by 60% at the higher paclitaxel concentration relative to the non-treated group and by 50% relative to the hyaluronic \acid gel group alone.

Table 1.

Treatment group	Individual ratings	Mean Severity of Adhesions	% of subjects >2.5
No Formulation (n=4)	3.0, 3.0, 3.5, 3.5	3.25	100%
Hyaluronic Acid Gel/Empty Micelles (n=5)	2.75, 2.0, 2.75, 3.0, 3.0	2.7	80%
Hyaluronic Acid Gel/Micellar Paclitaxel 0.6 mg (n=5)	1.25, 2.75, 3.5, 1.75, 2.5	2.35	60%
Hyaluronic Acid Gel/Micellar Paclitaxel 1.2 mg (n=5)	1.25, 1.25, 2.5, 1.75, 2.5	1.85	40%

CONCLUSION

These results suggest that micellar paclitaxel can improve preclinical surgical adhesion outcome in a well-established model of surgical adhesions. Such formulations can also reduce the incidence of surgical adhesions associated with cardiac surgery, when delivered through the intrapericardial route of administration.

EXAMPLE 12

INTRAPERICARDIAL PACLITAXEL DELIVERY INHIBITS NEOINTIMAL PROLIFERATION AND PROMOTES ARTERIAL ENLARGEMENT AFTER PORCINE CORONARY OVERSTRETCH

Catheter-based approaches to intrapericardial (IPC) delivery of therapeutic agents have been recently demonstrated to be feasible. This study examined the effect of IPC instillation of paclitaxel on neointimal proliferation induced by balloon overstretch of porcine coronary arteries.

METHODS

Both paclitaxel and copolymer were dissolved in 0.9% sodium chloride at 50°C±5°C. The solutions were then sterile-filtered and utilized within four hours for

pericardial instillation.

Eighteen juvenile female domestic pigs weighing 23 to 25 kg were used for this study. The animals were divided into three instillation groups: low-dose (LD, 10 mg paclitaxel, n=6); high-dose (HD, 50 mg paclitaxel, n=7); and control group (C, 50 mg copolymer, n=5). Each group had a consistent delivery volume (25 ml). All animals received a normal diet and were housed in similar runs.

EXPERIMENTAL PROTOCOL

All animals were fasted overnight and premedicated with aspirin (325 mg) 24 hours prior to operation. The animals were sedated with an intramuscular combination of ketamine (20 mg/kg), xylazine (2 mg/kg), and atropine (0.05 mg/kg). Anesthesia was initiated with IV sodium pentothal (25 mg/kg). After intubation, the animals were mechanically ventilated using air mixed with oxygen (2 L/min) and isoflurane (2.5%). The ECG and blood pressure were monitored continuously. The operation was carried out under sterile conditions.

All animals underwent coronary overstretch by balloon dilation. Briefly, access to the vascular system was obtained after cutdown of the right carotid or femoral artery. An 8F or 9F introducer sheath was inserted, followed by system heparinization (200 U/kg) and lidocaine (30 mg). An 8F guiding catheter was used to engage the left coronary artery. After intracoronary administered nitroglycerin (200 μg), a left coronary angiogram was performed. The cine frames were immediately converted to digital computer images and the LAD and LCx diameters determined using NIH Image, with the guiding catheter diameter serving as a reference length. A balloon catheter (20 mm long) with a 1.3 balloon: artery diameter ratio was chosen to dilate the target vessel. The balloon inflation was performed three times for 30 seconds, with a 60-second interval intervening. Coronary angiography was finally repeated, the catheters removed, and the cutdown was repaired.

PERCUTANEOUS INTRAPERICARDIAL SPACE DELIVERY

After balloon dilation, a pericardial access device was used for transthoracic insertion of a guidewire into the normal pericardial space. This device (PerDUCER®, Comedicus Inc. Columbia, MN) consists of a needle protectively sheathed with a catheter

bearing a hemispherical-shaped side-hole at its tip. An initial percutaneous tunnel was made below the xiphoid process using a 21-gauge needle introduced nearly parallel to the skin surface, after which a 0.038 inch diameter guidewire and introducer sheath were placed under fluoroscopic guidance into the mediastinum over the anterior pericardium. The sheathed needle device was inserted through an introducer sheath and positioned on the anterior outer surface of the pericardial sac, which was drawn into the hemispherical-shaped tip by manual suction and pierced by the needle. Finally, a 0.018 inch guidewire was placed through the needle lumen and advanced several cm to confirm confinement within the pericardial space. After removal of the needle, a 4F hydrophilic-coated dilator catheter was inserted over the wire. Following wire removal, successful intrapericardial tip placement was tested by contrast injection into the pericardial space; and twenty-five mls of either paclitaxel or copolymer solution was delivered over five minutes into the pericardial sac. The catheter was removed, the cutaneous puncture was sutured, and the animals allowed to recover.

At 28 days after the procedure, the animals were sedated and anesthetized, as previously described. The final coronary angiography was performed after heparin (200 U/kg) administration. The animals were then killed by a lethal dose of pentobarbital (65 mg/kg). Immediately after euthanasia, the heart and pericardial tissue were harvested, and the coronary arteries were perfusion-fixed with 10% buffered zinc formalin for 15 to 20 minutes at 80 mm Hg pressure.

TISSUE PREPARATION AND IMMUNOCYTOCHEMICAL STAINING

The pericardial cavity was inspected before the LAD and LCx were dissected from the heart. Gross pericardial space adhesion was quantified using a modification of the scoring system described by Hurewitz et al. The grades were assigned as: 0=normal, 1= focal thin adhesions, 2=diffuse widespread adhesions, 3=complete obliteration of the pericardial space. After paraffin embedding and sectioning, pericardial tissue was stained with hematoxylin-eosin and Masson's trichrome. Mesothelial cells on the parietal pericardium were graded as either absent or present. The thickness of the visceral pericardium was measured at four sites overlying the left and right atria and ventricles.

To examine the entire LAD and LCx vessel lengths, the vessels were sectioned at 3 mm intervals from the proximal to distal end, and embedded in paraffin using standard

histological techniques. Paraffin sections were cut at 6 μm, affixed to glass microscope slides, and stained with hematoxylin-eosin and Verhoeff-Van Gieson's reagents. Immunohistochemical analysis was performed on selected segments using primary antibodies including anti-smooth muscle α-actin (1:1000, Dako), von Willebrand factor (vWF, 1:600, Dako) and anti-matrix metalloproteinase antibodies (MMP-2, 1:100; Oncogene). Secondary antibody binding was revealed by avidin complex method, with a staining reaction performed using 3,3'-diaminobenzidine (DAB) solution (Sigma). Nuclei were counterstained with hematoxylin or methyl green. Endogenous peroxidase activity was blocked with 3% H_2O_2 solution for 5 minutes. Negative control stains were generated using nonimmune serum instead of primary antibody.

Apoptotic cells were detected by the Klenow fragment end labeling method, using a commercially available kit (Oncogene). Briefly, after deparaffinization, the tissue sections were treated with 20 μg/ml proteinase K/10 mM Tris/HCL, pH 8.0, for 10 minutes. After rinsing in 1×TBS, the Klenow labeling reaction mixture was added. In each experiment, a positive and negative control was included. The positive control was treated with DNaseI (1 mg/ml, 20 minutes, RT) to induce DNA strand breaks; the negative control was stained only with Klenow labeling reaction mix (without Klenow enzyme).

MORPHOMETRIC ANALYSIS

Morphometric measurements were performed using a light microscope (Olympus) at low power (x2.5 microscopic magnification) linked to a color video camera (Sony) and a computer-interfaced image analysis system with NIH Image software. This allowed the manual selection and delineation of artery areas. The endoluminal length (ELL), the circumference bounded by internal elastic lamina (IEL) and the external elastic lamina (EEL) were traced by hand, and the luminal and intimal areas were determined automatically. Fracture length (FL) was defined as the arc length between the two fracture points of the internal elastic lamina. Intimal area (IA) was measured directly. Maximal intimal thickness (MIT) was defined as the maximal distance between the lumen and EEL, while maximal adventitial thickness (MAT) was the analogous length between EEL and adventitia. The percent stenosis (%) was described as the histologic lumen diameter at the site of maximal stenosis divided by the pre-angioplasty luminal diameter determined at the midpoint of the

target segment.

CELL COUNTING

Analysis was done on each cross-section with hematoxylin-stained nuclei of neointima and media cells under ×40 microscopic magnification. Neointima and media cells were counted using a validated method. Briefly, randomly selected areas encompassing 20% to 40% of the total neointimal cross-sectional area were counted. The cells within the media were counted in five regions defined as follows: region 1 and 2, comprised of the two medial ends adjacent to the medial tear; region 3, the site 180° opposite to the neointimal mass; and regions 4 and 5, at 90° radials with respect to the neointima. Cell density (cells/mm^2) was used to determine total numbers in the neointimal and medial areas.

Apoptotic cells in the neointima or media were scored as positive when showing morphologic features characteristic of apoptosis as well as positive Klenow labeling. Cells with cytoplasmic, but not nuclear staining, were scored as negative cells. The apoptosis percentage was determined for each vessel layer.

STATISTICS

Results are presented as mean±SEM. An unpaired *t*-test was used to compare the three group histomorphometric measurement data. Differences are considered significant at $p < 0.05$. All statistical calculations were performed using the SigmaStat™ software package.

RESULTS

Pericardial instillation was well tolerated by all animals. No complications developed during these procedures, and no clinical evidence of paclitaxel-related toxicity was noted.

BASELINE ANGIOGRAPHIC CHARACTERISTICS

The artery diameters before balloon dilation (C, 2.65±0.12 mm; LD, 2.51±0.13 mm; HD, 2.47±0.12 mm; p = NS) and the balloon/artery ratios (C, 1.34±0.02; LD, 1.32±0.03; HD, 1.32±0.02; p = NS) were no different among control, high-dose, and low-dose groups.

PERICARDIAL TISSUE AND CONTENTS

The gross and histological changes of the pericardium after IPC paclitaxel delivery are summarized in Table 1. Intrapericardial adhesions were entirely absent in the C and LD groups, except for three pigs with a few thin adhesions limited to the puncture site (1 pig in control, 2 pigs in LD group). Biochemical parameters were measured in the pericardial fluid of a subset of the LD and C groups, and were within normal ranges. Macroscopic scoring of the pericardial space adhesions, confirmed that the high-dose, but not the low-dose group, was significantly different from control (Adhesion scores C, 0.20±0.20; LD, 0.33±0.21; and HD, 2.57±0.20; $p < .001$ for HD vs. C or LD). Microscopically, the C and LD groups had entirely intact mesothelial layers, with multilayering noted in some parts of the pericardium. In addition to the intracavitary adhesions, the pericardium of the HD group was demonstrably thicker as compared to both the C and LD groups, with an increase found in the dense connective tissue of both parietal and visceral layers. The interlaminar adhesion tissue displayed fibrin and collagen deposition as well as infiltration with mononuclear cells. Cells staining positively for SMC α-actin expression were found dispersed throughout the connective tissue of the visceral pericardium in all groups and displayed greatest intensity of staining in the high-dose group.

MORPHOMETRIC ANALYSIS OF ARTERIES

IPC paclitaxel delivery significantly inhibited neointimal proliferation. Table 2 displays morphometric data for vessels comprising each group. The extent of vessel injury, expressed as an injury index (FL/FL+IEL) was equivalent for each of the three groups (C, 0.21±0.02; LD, 0.22±0.03; and HD, 0.21±0.01; p = NS). The neointimal response was linearly correlated with the degree of vessel injury in the control as well as the experimental groups (Fig. 7, C, $R^2 = 0.69$; HD, $R^2 = 0.66$; LD, $R^2 = 0.44$), but the slope is markedly diminished for vessels receiving paclitaxel at either dose. The absolute neointimal area (Fig. 8A) was significantly smaller in both experimental groups (LD, 0.47±0.04 and HD, 0.51±0.06 mm^2, p = NS) when compared with the control group (0.79±0.07 mm^2, $p < .001$). The neointimal area normalized to FL was also significantly smaller for both treatment groups (LD, 0.32±0.02 and HD, 0.39±0.04) than in the control group (0.68±0.03, $p < .001$). Similarly, the maximal intimal and adventitial thicknesses were lower in both paclitaxel

groups than the C group ($p < .001$). However, the medial area did not differ among the groups. Both paclitaxel groups evidenced outward vascular remodeling in comparison with the control group, with the external elastic lamina (EEL) circumference and enclosed area found to be significantly larger in the LD and HD group (Table 2).

The degree of luminal occlusion, expressed as % stenosis, was significantly reduced in both treated groups (LD, 10±0.02%; and HD, 22±0.03%; versus C 38±0.03%, $p < .001$) (Fig. 8B)), for relative stenosis reductions of 74% in the LD and 42% in the HD groups. The marked reduction in luminal occlusion by paclitaxel is dominantly achieved by its effects on vessel remodeling as expressed in terms of EEL circumference. Comparative evaluation of the area contributions of the decreased neointima and the increased vessel circumference shows that the latter is in fact responsible for 70-80 % of the luminal expansion noted relative to control vessels.

CELL QUANTIFICATION AND IMMUNOHISTOCHEMISTRY

Medial cell density was not measurably different among the C (3983±128 cells/mm^2), LD (3875±244 cells/mm^2), and HD (4089±422 cells/mm^2) groups. However, the neointimal cell density in the HD group (3571±128 cells/mm^2) was significantly lower than C (4574±201 cells/mm^2) and LD (4196±120 cells/mm^2) groups ($p < .001$). The Klenow-positive cells were predominately detected in the neointima, with very few cells found in the media or adventitia. Most staining cells also demonstrated hyperchromatic and fragmented nuclei. Some had histologically normal nuclei, possibly representing cells in an early apoptotic phase. There were no significant differences between the C and LD groups, but the HD group had significantly greater percentage of apoptotic cells than either of these groups (C, 1.72%; LD, 2.31%; versus HD, 7.07%; $p < .0001$).

Immunohistochemical staining demonstrated that neointimal cells were predominantly immunoreactive for α-actin in all groups. The neointima was composed of spindle-shaped cells and a large amount of loose extracellular matrix, where the cells had the appearance of a synthetic phenotype with looser organization than found in media. At 28 days after balloon injury, complete vessel re-endothelialization had been achieved in most vessel segments of all three groups, as measured by positive vWF staining. Since paclitaxel has been known to alter the expression of MMP-2 in other systems, we investigated the

presence of such modulation following IPC paclitaxel delivery. In control, as well as LD vessels, MMP-2 immunoreactivity was found in endothelial cells, neointima, and media. Conversely, there was generally diminished MMP-2 staining in all vessel layers in the HD sections. MMP-2 staining was not characteristically present in the adventitia of any groups.

The present study demonstrates that a single-dose of paclitaxel delivered into the pericardial space results in significant reduction of restenosis after balloon injury of the porcine coronary artery, which is mediated both by reduced neointimal formation and enhanced arterial enlargement. The 10 mg dosage appeared to produce more optimal results, although favorable effects were present at both doses. The clear effect identified *in vivo* 28 days after a single dose is remarkable, and likely may relate to the high affinity of paclitaxel for its specific intracellular target sites on microtubules, as well as its hydrophobic nature, both of which will favor slow redistribution following placement at a particular site. The fluid reservoir formed by the pericardial sac would also be expected to contribute to the persistence of locally effective concentrations of agents delivered by an IPC route.

The vessel circumference evaluated at the external elastic lamina becomes remarkably larger following paclitaxel therapy (C, 7.04±0.22; versus HD, 7.71±0.23; and LD, 8.12±0.18 mm, $p = .017$). Such an effect, found in the context of a conserved medial area, reflects a modulated disposition of tissue mass consistent with altered vascular remodeling in comparison with the control group. The associated finding of reduced adventitial thickness in this study provokes the hypothesis that a decrease in adventitial fibrosis may contribute in part to this positive remodeling response. The net result of the increment in vessel circumference and the reduction in neointimal mass due to IPC paclitaxel is an increase in luminal size from C, 5.12±0.23 to HD, 6.15±0.25, and LD, 7.02±0.18 mm, ($p = 0.002$, 0.006 respectively). This dual effect of paclitaxel on remodeling and proliferation is encouraging since multiple studies have suggested that both increased total SMC bulk and vascular remodeling are key contributors to restenosis after angioplasty, while numerous therapeutic agents affecting predominantly SMCs proliferation have been found insufficient to prevent vessel renarrowing.

MMPs and their inhibitors, which regulate extracellular matrix homeostasis, might play a significant role in normal and pathologic vessel remodeling. Degradation of the

elastic laminae by MMP-2 is accentuated in both inward remodeling due to low flow and outward remodeling due to high flow, and appears to be a necessary component of any lasting structural modification of the vessel wall.

MMP-2 expression was clearly detectable in the C and LD groups following porcine coronary angioplasty, but was generally lost or reduced in the HD vessel segments. The mechanism of MMP-2 downregulation following exposure to paclitaxel at the 50 mg dose is not clear. The presence of MMP-2 immunoreactivity in the C and LD groups must be interpreted with caution because of the absence of data confirming zymogen activation and molar excess with respect to tissue metalloprotease inhibitor (TIMP) levels, a limitation of this study. Nevertheless, the absence of MMP-2 staining in the HD group clearly suggests the lack of such activity in these specimens. This, in turn, generates the hypothesis that the loss of MMP-2 is linked to the diminished outward remodeling found in the HD group.

IPC paclitaxel treatment at both selected doses does not appear to be causing overt damage to either the endothelial or medial layers. Endothelial regeneration was present to a nearly complete degree in all groups. Likewise, medial cell densities and areas were no different among the three groups.

The diminished outward remodeling in the 50 mg dosage group is largely responsible for partial loss of the anti-stenotic effect seen upon administration of the lower dose of drug. This biphasic dose-response may be interpreted as defining the transition into a supra-therapeutic drug level for this delivery modality.

CONCLUSIONS

A single-dose perivascular delivery of paclitaxel into the pericardial space significantly preserves luminal patency in the porcine coronary balloon overstretch model. The mechanism by which IPC delivery of paclitaxel maintains vascular lumen area involves promotion of positive vascular remodeling as well as inhibition of SMC hyperplasia. This study further establishes a maximum dose for IPC paclitaxel using the polymeric formulation described, and suggests that a carefully chosen dose of paclitaxel can be employed for the inhibition of post-angioplasty restenosis via IPC delivery.

Table 1. Histologic Changes in Pericardium 28 Days after Paclitaxel Delivery

	Adhesion score	Mesothelium Intact/Total	Visceral thickness (mm)
Control	0.20±0.20	5/5	0.22±0.02
Low-dose	0.33±0.21	6/6	0.23±0.03
High-dose	2.57±0.20*	3/7	0.45±0.02*

Values are Mean ± SEM.
Mesothelium indicates the number of pigs with mesothelium layer divided by each group total pigs.
* $p < .001$ versus control and low-dose.

Table 2. Morphometric Analysis of the Effect of IPC Paclitaxel Delivery

	Control	High-dose	Low-dose	*P*
FL/FL+IEL	0.21±0.02	0.22±0.03*	0.21±0.01*	* = NS
Intimal area (IA, mm^2)	0.79±0.07	0.51±0.06*	0.47±0.04*	* < .001
IA/FL	0.68±0.03	0.39±0.04*	0.32±0.02*°	* < .001; °.043
Maximal intima thickness(mm)	0.56±0.02	0.42±0.03*	0.43±0.03*	* < .001
Media area (MA, mm^2)**	1.07±0.07	1.15±0.10*	1.13±0.05*	* = NS
Maximal adventitial thickness(mm)	0.47±0.02	0.38±0.03*	0.35±0.02*	* < .001
External lamina circumference (mm)	7.04±0.22	7.71±0.23*	8.12±0.18*°	* = .001; °.017
External lamina area (mm^2)**	3.94±0.18	4.73±0.19*	5.25±0.20*°	* = .001; °.017
Endoluminal circumference (mm)	5.12±0.23	6.15±0.25*	7.02±0.18*°	* = .002; °.006

Values are Mean ± SEM.
FL indicates fracture length. IEL indicates internal elastic lamina length.
* versus control, ° low-dose versus high-dose.
** Area values for these parameters determined from circumference values using circular geometry.

EXAMPLE 13

TREATMENT OF RESTENOSIS IN RELATION TO VASCULAR GRAFT

MATERIALS AND METHODS:

The experiment involved implantation of a vascular graft between the common carotid artery and the external jugular vein in pigs (4 mm graft, n=26; 6 mm graft, n=19).

However, graft patency could not consistently be maintained due to a high frequency of graft thrombosis (75% in 4mm grafts and 50% in 6mm grafts). Thus, we developed a pig model of arterio-venous (AV) fistula where the end of the internal jugular vein was connected to the side of the common carotid artery (n=12). Pigs weighing 40 kg to 50 kg were anesthetized with 1.5% halothane after sedation with ketamine. A vertical incision was made on the right side of the trachea, and the right common carotid artery and internal jugular vein were exposed. Blood flow in the vein was interrupted proximally with a vascular clamp. The vein was tied off distally and cut. Blood flow in the artery was stopped with two vascular clamps and an arteriotomy made between the clamps. The free end of the vein was sutured to the artery with 6-0 Proline. Blood flow was restored by removing all three clamps. Bleeding at the anastomosis was controlled by temporary application of Gelfoam and patency of the fistula was checked. A 4 cm x 6 cm paclitaxel loaded (20% or 5%) EVA film or a control EVA film devoid of drug was applied around the anastomosis. The wound was closed. The same procedure was performed on the left side but the anastomosis was not treated so that each animal could be used as its own control. Two pigs were used in each group. After 28 days, the animals were sacrificed and pressure perfused at 100 mmHg with 10% buffered formaldehyde. Both AV fistulae were harvested. Cross-sections were cut within the anastomosis and in the artery and vein. Sections were stained with hematoxylin-and-eosin and Movat's stains and the effect of paclitaxel on venous stenosis was assessed.

RESULTS:

Untreated AV fistulae exhibited a pronounced venous intimal thickening (Figure 9B and 10B). The neointima exhibited numerous cells and the extracellular matrix was rich in collagen and proteoglycan. Intimal hyperplasia was inhibited in animals treated with 5% and 20% paclitaxel (Figure 9A and 10A). It was not affected by control EVA films devoid of paclitaxel. Similarly to Experiment I in rats, an acellular fibrin layer was present around AV fistulae treated with paclitaxel (Figure 9A and 10A).

CONCLUSION

Perivascular paclitaxel slowly released from EVA films prevents venous intimal hyperplasia after creation of an arterio-venous shunt. The presence of the

periadventitial fibrin layer suggests that the treated anastomoses are stronger than the control ones. This technology should extend the life of vascular grafts in humans by reducing venous stenosis, the principal cause of graft failure.

EXAMPLE 14

EFFECT OF ANTI-MICROTUBULE AGENTS ON NEUTROPHIL ACTIVITY

The example describes the effect of anti-microtubule agents on the response of neutrophils stimulated with opsonized CPPD crystals or opsonized zymosan. As shown by experiments set forth below, anti-microtubule agents are strong inhibitors of particulate-induced neutrophil activation as measured by chemiluminescence, superoxide anion production and degranulation in response to plasma opsonized microcrystals or zymosan.

MATERIALS AND METHODS

Hanks buffered saline solution (HBSS) pH 7.4 was used throughout this study. All chemicals were purchased from Sigma Chemical Co (St. Louis, MO) unless otherwise stated. All experiments were performed at 37°C unless otherwise stated.

1. PREPARATION AND CHARACTERIZATION OF CRYSTALS

CPPD (triclinic) crystals were prepared. The size distribution of the crystals was approximately 33% less than 10 μm, 58% between 10 and 20 μm and 9% greater than 20 μm. Crystals prepared under the above conditions are pyrogen-free and crystals produced under sterile, pyrogen-free conditions produced the same magnitude of neutrophil response as crystals prepared under normal, non-sterile laboratory conditions.

2. OPSONIZATION OF CRYSTALS AND ZYMOSAN

All experiments that studied neutrophil responses to crystals or zymosan in the presence of paclitaxel were performed using plasma opsonized CPPD or zymosan. Opsonization of crystals or zymosan was done with 50% heparinized plasma at a concentration of 75 mg of CPPD or 12 mg of zymosan per ml of 50% plasma. Crystals or zymosan were incubated with plasma for 30 minutes at 37°C and then washed in excess HBSS.

3. NEUTROPHIL PREPARATION

Neutrophils were prepared from freshly collected human citrated whole blood. Briefly, 400 ml of blood were mixed with 80 ml of 4% dextran T500 (Phamacia LKB, Biotechnology AB Uppsala, Sweden) in HBSS and allowed to settle for 1 hour. Plasma was collected continuously and 5 ml applied to 5 ml of Ficoll Paque (Pharmacia) in 15 ml polypropylene tubes (Corning, NY). Following centrifugation at 500 g for 30 minutes, the neutrophil pellets were washed free of erythrocytes by 20 seconds of hypotonic shock. Neutrophils were resuspended in HBSS, kept on ice and used for experiments within 3 hours. Neutrophil viability and purity was always greater than 90%.

4. INCUBATION OF NEUTROPHILS WITH ANTI-MICROTUBULE AGENTS

(a) Paclitaxel

A stock solution of paclitaxel at 12 mM in dimethylsulfoxide (DMSO) was freshly prepared before each experiment. This stock solution was diluted in DMSO to give solutions of paclitaxel in the 1 to 10 mM concentration range. Equal volumes of these diluted paclitaxel solutions was added to neutrophils at 5,000,000 cells per ml under mild vortexing to achieve concentrations of 0 to 50 μM with a final DMSO concentration of 0.5%. Cells were incubated for 20 minutes at 33°C then for 10 minutes at 37°C before addition to crystals or zymosan.

(b) Aluminum Fluoride

A stock solution of aluminum fluoride (AlF_3) at 1 M in HBSS was freshly prepared. This stock solution was diluted in HBSS to give solutions of AlF_3 in the 5 to 100 mM concentration range. Equal volumes (50 μl) of these diluted AlF_3 solutions was added to neutrophils at 5,000,000 cells per ml and incubated for 15 minutes at 37°C. Luminol (1 μM) was added and then 20 μl of opsonized zymosan (final concentration = 1 mg/ml) to activate the cells.

(c) Glycine Ethyl Ester

A stock solution of glycine ethyl ester at 100 mM in HBSS was freshly prepared. This stock solution was diluted in HBSS to give solutions of glycine ethyl ester in

the 0.5 to 10 mM concentration range. Equal volumes (50 μl) of these diluted glycine ethyl ester solutions was added to neutrophils at 5,000,000 cells per ml and incubated for 15 minutes at 37°C. Luminol (1 μM) was added and then 20 μl of opsonized zymosan (final concentration = 1 mg/ml) to activate the cells.

(d) LY290181

A stock solution of LY290181 at 100 μM in HBSS was freshly prepared. This stock solution was diluted in HBSS to give solutions of LY290181 in the 0.5 to 50 μM concentration range. Equal volumes (50 μl) of these diluted LY290181 solutions was added to neutrophils at 5,000,000 cells per ml and incubated for 15 minutes at 37°C. Luminol (1 μM) was added and then 20 μl of opsonized zymosan (final concentration = 1 mg/ml) to activate the cells.

5. CHEMILUMINESCENCE ASSAY

All chemiluminescence studies were performed at a cell concentration of 5,000,000 cells/ml in HBSS with CPPD (50 mg/ml). In all experiments 0.5 ml of cells was added to 25 mg of CPPD or 0.5 mg of zymosan in 1.5 ml capped Eppendorf tubes. 10 μl of luminol dissolved in 25% DMSO in HBSS was added to a final concentration of 1 μM and the samples were mixed to initiate neutrophil activation by the crystals or zymosan. Chemiluminescence was monitored using an LKB Luminometer (Model 1250) at 37°C for 20 minutes with shaking immediately prior to measurements to resuspend the crystals or zymosan. Control tubes contained cells, drug and luminol (crystals absent).

6. SUPEROXIDE ANION GENERATION

Superoxide anion concentrations were measured using the superoxide dismutase inhibitable reduction of cytochrome C assay. Briefly, 25 mg of crystals or 0.5 mg of zymosan was placed in a 1.5 ml capped Eppendorf tube and warmed to 37°C. 0.5 ml of cells at 37°C were added together with ferricytochrome C (final concentration 1.2 mg/ml) and the cells were activated by shaking the capped tubes. At appropriate times tubes were centrifuged at 10,000g for 10 seconds and the supernatant collected for assay be measuring the absorbance of 550 nm. Control tubes were set up under the same conditions with the inclusion of superoxide dismutase at 600 units per ml.

7. NEUTROPHIL DEGRANULATION ASSAY

One and a half milliliter Eppendorf tubes containing either 25 mg of CPPD or 1 mg of zymosan were preheated to 37°C. 0.5 ml of cells at 37°C were added followed by vigorous shaking to initiate the reactions. At appropriate times, tubes were centrifuged at 10,000 g for 10 seconds and 0.4 ml of supernatant was stored at -20°C for later assay.

Lysozyme was measured by the decrease in absorbance at 450 nm of a *Micrococcus lysodeikticus* suspension. Briefly, *Micrococcus lysodeikticus* was suspended at 0.1 mg/ml in 65 mM potassium phosphate buffer, pH 6.2 and the absorbance at 450 nm was adjusted to 0.7 units by dilution. The crystal (or zymosan) and cell supernatant (100 µl) was added to 2.5 ml of the *Micrococcus* suspension and the decrease in absorbance was monitored. Lysozyme standards (chicken egg white) in the 0 to 2000 units/ml range were prepared and a calibration graph of lysozyme concentration against the rate of decrease in the absorbance at 450 nm was obtained.

Myeloperoxidase (MPO) activity was measured by the increase in absorbance at 450 nm that accompanies the oxidation of dianisidine. 7.8 mg of dianisidine was dissolved in 100 ml of 0.1 M citrate buffer, pH 5.5 at 3.2 mM by sonication. To a 1 ml cuvette, 0.89 ml of the dianisidine solution was added, followed by 50 µl of 1% Triton x 100, 10 µl of a 0.05% hydrogen peroxide in water solution and 50 µl of crystal-cell supernatant. MPO activity was determined from the change in absorbance (450 nm) per minute, Delta Å 450, using the following equation:

Dianisidine oxidation (nmol/min) = 50 x Delta Å 450

8. NEUTROPHIL VIABILITY

To determine the effect of the anti-microtubule agents on neutrophil viability the release of the cytoplasmic marker enzyme, lactate dehydrogenase (LDH) was measured. Control tubes containing cells with drug (crystals absent) from degranulation experiments were also assayed for LDH.

B. Results

In all experiments statistical significance was determined using Students' t-test and significance was claimed at $p<0.05$. Where error bars are shown they describe one

standard deviation about the mean value for the n number given.

1. NEUTROPHIL VIABILITY

(a) Paclitaxel

Neutrophils treated with paclitaxel at 46 µM for one hour at 37°C did not show any increased level of LDH release (always less than 5% of total) above controls indicating that paclitaxel did not cause cell death.

(b) Aluminum Fluoride

Neutrophils treated with aluminum fluoride at a 5 to 100 mM concentration range for 1 hour at 37°C did not show any increased level of LDH release above controls indicating that aluminum fluoride did not cause cell death.

(c) Glycine Ethyl Ester

Neutrophils treated with glycine ethyl ester at a 0.5 to 20 mM concentration range for 1 hour at 37°C did not show any increased level of LDH release above controls indicating that glycine ethyl ester did not cause cell death.

2. CHEMILUMINESCENCE

(a) Paclitaxel

Paclitaxel at 28 µM produced strong inhibition of both plasma opsonized CPPD and plasma opsonized zymosan-induced neutrophil chemiluminescence. The inhibition of the peak chemiluminescence response was 52% (+/-12%) and 45% (+/-11%) for CPPD and zymosan respectively. The inhibition by paclitaxel at 28 µM of both plasma opsonized CPPD and plasma opsonized zymosan-induced chemiluminescence was significant at all times from 3 to 16 minutes. These experiments show the concentration dependence of paclitaxel inhibition of plasma opsonized CPPD-induced neutrophil chemiluminescence. In all experiments control samples never produced chemiluminescence values of greater than 5 mV and the addition of paclitaxel at all concentrations used in this study had no effect on the chemiluminescence values of controls.

(b) Aluminum Fluoride

Aluminum fluoride at concentrations of 5 to 100 mM produced strong inhibition of plasma opsonized zymosan-induced neutrophil chemiluminescence, in a concentration dependent manner (concentration dependence of AlF_3 inhibition of plasma opsonized zymosan-induced neutrophil chemiluminescence). The addition of AlF_3 at all concentrations used in this study had no effect on the chemiluminescence values of controls.

(c) Glycine Ethyl Ester

Glycine ethyl ester at concentrations of 0.5 to 20 mM produced strong inhibition of plasma opsonized zymosan-induced neutrophil chemiluminescence (glycine ethyl ester inhibition of plasma opsonized zymosan-induced neutrophil chemiluminescence was concentration dependent). The addition of glycine ethyl ester at all concentrations used in this study had no effect on the chemiluminescence values of controls.

(d) LY290181

LY290181 at concentrations of 0.5 to 50 μM produced strong inhibition of plasma opsonized zymosan-induced neutrophil chemiluminescence. The addition of LY290181 at all concentrations used in this study had no effect on the chemiluminescence values of controls.

3. SUPEROXIDE GENERATION

Plasma opsonized CPPD crystal-induced superoxide anion production, as measured by the superoxide dismutase (SOD) inhibitable reduction of cytochrome C. In particular, treatment of the cells with paclitaxel at 28 μM produced a decrease in the amount of superoxide generated at all times. This decrease was significant at all times. Stimulation of superoxide anion production by opsonised zymosan showed a similar time course to CPPD-induced activation. The inhibition of zymosan-induced superoxide anion production by paclitaxel at 28 μM was less dramatic than the inhibition of CPPD activation but was significant at all times tested

Treatment of CPPD crystal-induced neutrophils with LY290181 at 17 μM also produced a decrease in the amount of superoxide generated.

4. NEUTROPHIL DEGRANULATION

Neutrophil degranulation was monitored by the plasma opsonized CPPD crystal-induced release of myeloperoxidase and lysozyme or the plasma opsonized zymosan-induced release of myeloperoxidase. It has been shown that sufficient amounts of these two enzymes are released into the extracellular media when plasma coated CPPD crystals are used to stimulate neutrophils without the need for the addition of cytochalasin B to the cells.

Paclitaxel at 28 μM reduced lysozyme release and this inhibition of degranulation was significant at all times tested.

Only minor amounts of MPO and lysozyme were released when neutrophils were stimulated with opsonized zymosan. Despite these low levels it was possible to monitor 50% inhibition of MPO release after 9 minutes incubation in the presence of paclitaxel at 28 μM that was statistically significant ($p<0.05$) (data not shown). Treatment of CPPD crystal-induced neutrophils with LY290181 at 17 μM decreased both lysozyme and myeloperoxidase release from the

C. Discussion

These experiments demonstrate that paclitaxel and other anti-microtubule agents are strong inhibitors of crystal-induced neutrophil activation. In addition, by showing similar levels of inhibition in neutrophil responses to another form of particulate activator, opsonized zymosan, it is evident that the inhibitory activity of paclitaxel and other anti-microtubule agents are not limited to neutrophil responses to crystals. Paclitaxel, aluminum fluoride, glycine ethyl ester and LY290181 were also shown to be strong inhibitors of zymosan-induced neutrophil activation without causing cell death. LY290181 was shown to decrease superoxide anion production and degranulation of CPDD crystal-induced neutrophils.

EXAMPLE 15

T CELL RESPONSE TO ANTIGENIC STIMULUS

In order to determine whether paclitaxel affects T-cell activation in response to

stimulagens, TR1 T-cell clones were stimulated with either the myelin basic protein peptide, GP68-88, or the lectin, conA, for 48 hours in the absence or presence of increasing concentrations of paclitaxel in a micellar formulation. Paclitaxel was added at the beginning of the experiment or 24 hours following the stimulation of cells with peptide or conA. Tritiated thymidine incorporation was determined as a measure of T-cell proliferation in response to peptide or conA stimulation.

The results demonstrated that T-cell stimulation increased in response to the peptide GP68-88 and conA. In the presence of control polymeric micelles, T-cell stimulation in response to both agonists was not altered. However, treatment with paclitaxel micelles, either at the beginning of the experiment or 24 hours following the stimulation, decreased T-cell response in a concentration dependent manner. Under both conditions, T-cell proliferation was completely inhibited by 0.02 μM paclitaxel

These data indicate that paclitaxel is a potent inhibitor of T-cell proliferation in response to antigen-induced stimulation.

From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the invention is not limited by the specific examples provided herein.

CLAIMS

1. A method for treating or preventing disease of the pericardium, heart, or coronary vasculature, comprising administering intrapericardially to a patient an anti-microtubule agent, such that said disease of the pericardium, heart, or coronary vasculature is treated or prevented.

2. The method according to claim 1 wherein said anti-microtubule agent is paclitaxel, or an analogue or derivative thereof.

3. The method according to claim 1 wherein said disease is intimal hyperplasia.

4. The method according to claim 1 wherein said anti-microtubule agent further comprises a polymer.

5. The method according to claim 1 wherein said polymer is poly-lactic acid.

6. The method according to claim 1 wherein said polymer is hyaluronic acid.

7. The method according to claim 5 wherein said biodegradable polymer is comprised of poly(hydroxy acid), poly (lactones), poly (amino acids), poly (anhydrides), poly (orthoesters), poly (phosphazines), poly (phosphesters), poly saccharrides, and the co-polymers and blends of any of these.

8. The method according to claim 1 wherein said polymer is poly-lactic acid.

9. The method according to claim 1 wherein said polymer is hyaluronic

acid.

10. The method according to claim 1 wherein said disease is stenosis, restenosis, or in-stent restenosis.

11. The method according to claim 1 wherein said disease is atherosclerosis.

12. The method according to claim 1 wherein said disease is transplant rejection.

13. The method according to claim 1 wherein said disease is arteritis.

14. The method according to claim 1 wherein said disease is a rheumatic condition affecting the heart.

15. The method according to claim 1 wherein said disease is valvular stenosis.

16. The method according to claim 1 wherein said disease is shunt restenosis.

17. The method according to claim 1 wherein said disease is cardiac adhesion.

18. The method according to claim 1 wherein said disease is a malignant pericardial effusion.

19. The method according to claim 1 wherein said disease is a cardiac rhythm disorder.

Figure 1

Intrapericardial Micellar Paclitaxel - Treatment Protocol

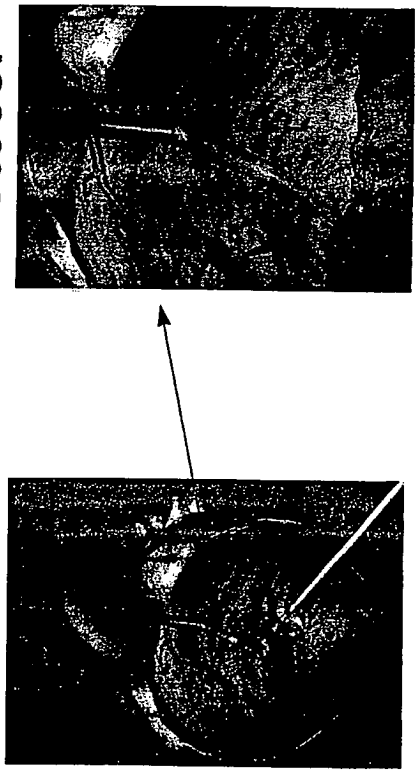


Figure 2

Fracture Length 28 Days Following Balloon Injury in Swine Coronary Arteries

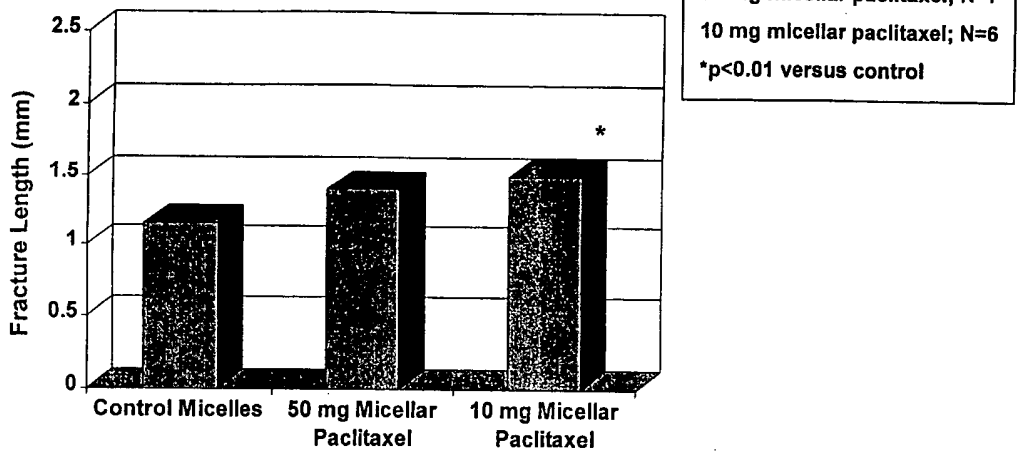


Figure 3

Intrapericardial Micellar Paclitaxel Reduces Calculated Diameter Coronary Stenosis

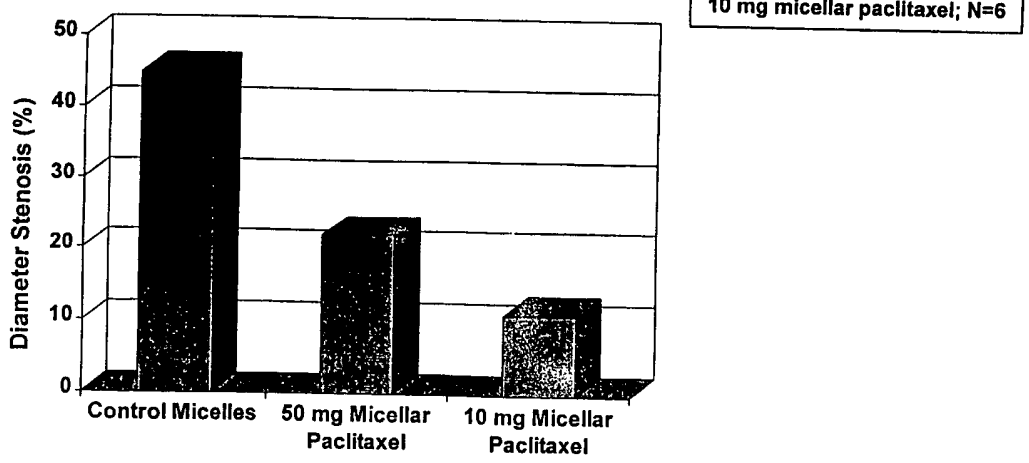


Figure 4

Intrapericardial Micellar Paclitaxel

(Swine Coronary Artery Balloon Injury Model)

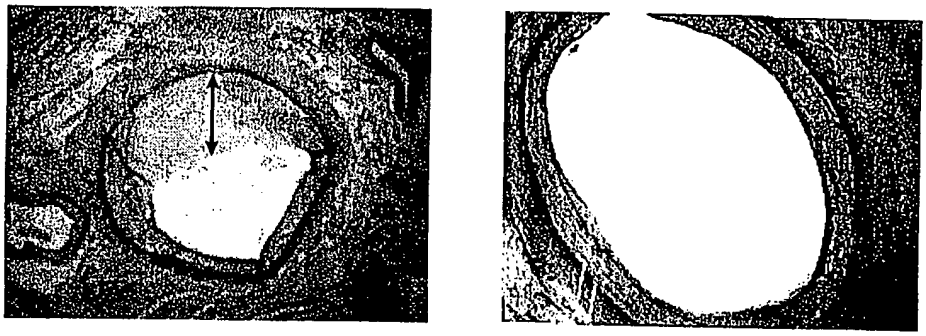


Figure 5

Intrapericardial Micellar Paclitaxel Results in Significantly Reduced Neointimal Area

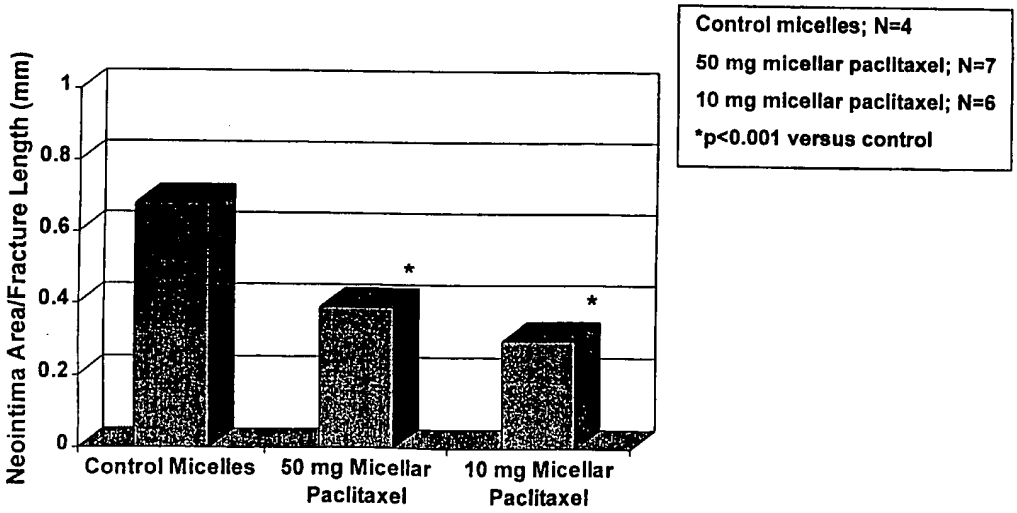
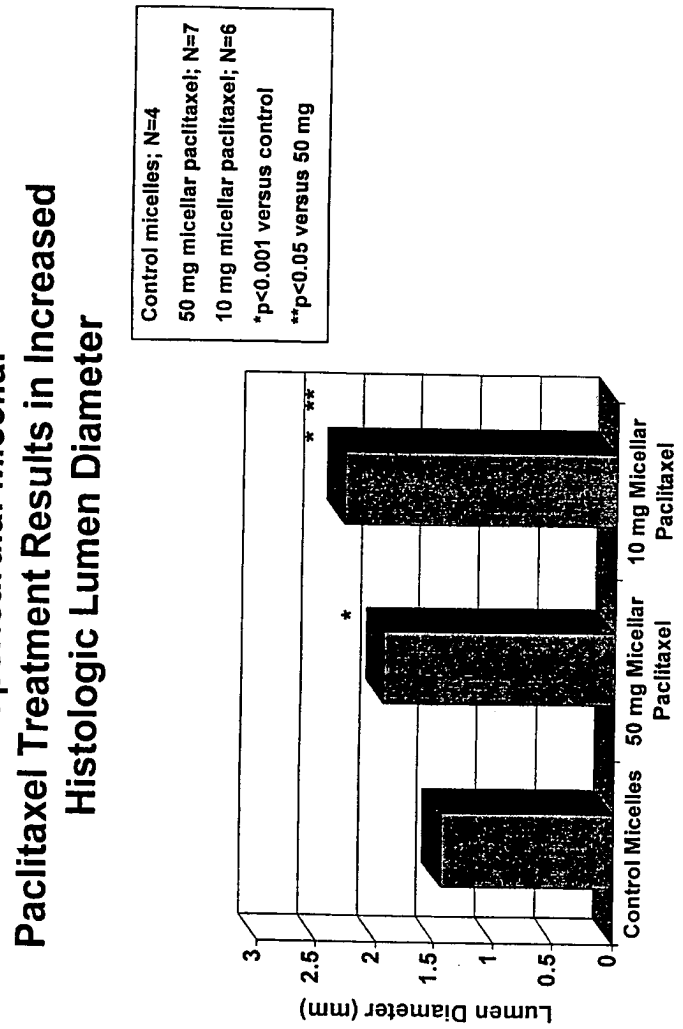


Figure 6



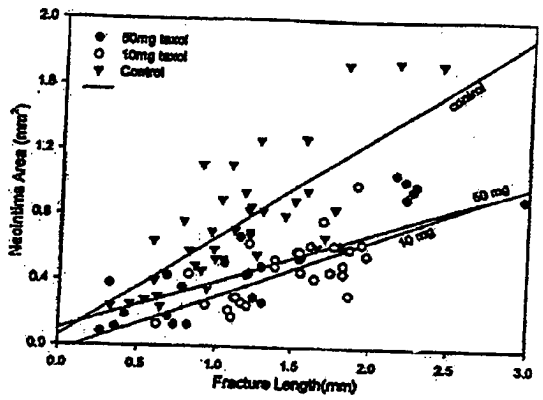


Figure 1: Graph of effect of IP delivery of paclitaxel on vessel response to overstretch injury. The R^2 values for the linear regression analysis control, HD, and LD were 0.69, 0.66 and 0.44, respectively. A positive effect is presented by treated groups, which show bigger fracture length, smaller neointimal area.

Figure 7

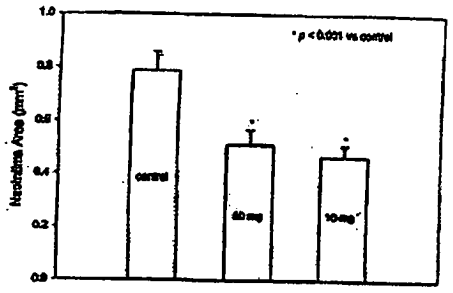


Figure 8A

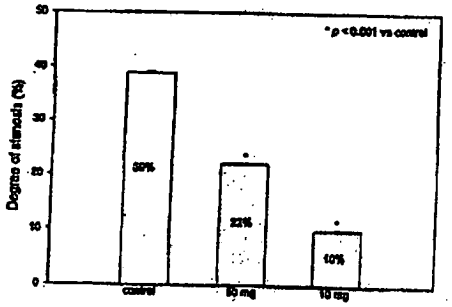
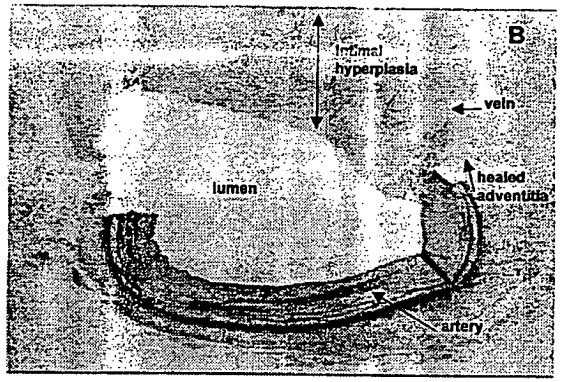
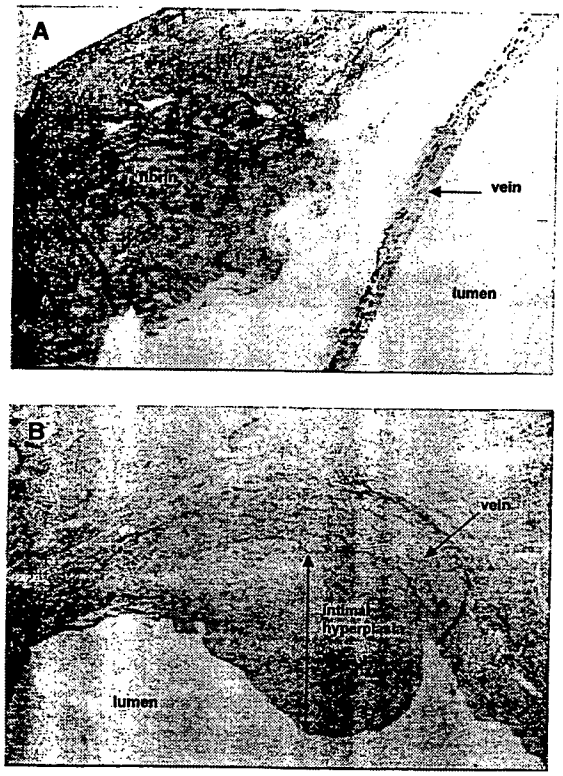


Figure 8B

Figure 2: Results of morphometric data of IP delivery after 28 days. (A) and (B) compares neointimal area and calculated diameter of stenosis between three groups, respectively. * indicates the values of $p<.001$ vs control.



FIGURES 9A and 9B



FIGURES 10A and 10B

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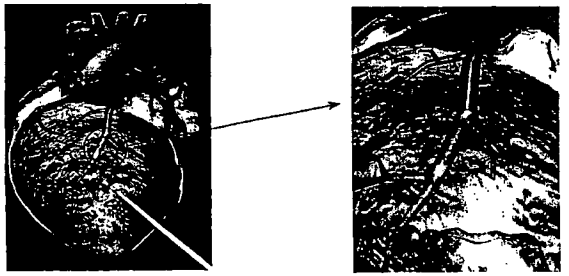
[Continued on next page]

(54) Title: INTRA-PERICARDIAL DELIVERY OF ANTI-MICROTUBULE AGENTS

Intrapericardial Micellar Paclitaxel - Treatment Protocol



(57) Abstract: Methods and compositions are provided for intra-pericardial administration of anti-microtubule agents, suitable for use in treating or preventing a variety of diseases of the pericardium, heart, or, coronary vasculature.

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B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, CHEM ABS Data, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SUH H ET AL: "Regulation of smooth muscle cell proliferation using paclitaxel-loaded poly(ethylene oxide)-poly(lactide/glycolide) nanospheres." JOURNAL OF BIOMEDICAL MATERIALS RESEARCH, (1998 NOV) 42 (2) 331-8., XP000937874 the whole document ---	1-10,15, 16
X	WO 97 33552 A (LI CHUN ;WALLACE SIDNEY (US); YU DONG FANG (US); WALLACE TECH INC) 18 September 1997 (1997-09-18) page 9, line 11 -page 10, line 5 page 15, line 13-27 claims 1,12,13,36-40; examples 2,3 --- -/--	1-5,7,8, 10,15,16

[X] Further documents are listed in the continuation of box C. [X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 September 2000	23.03.01

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Veronese, A

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Int. ...ional Application No
PCT/US 00/02376

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 25176 A (RENO JOHN M ;KUNZ LAWRENCE L (US); NEORX CORP (US)) 22 August 1996 (1996-08-22) claims 1,2,4,8,9,11,18-22,27-30,36-43,49-51,53-55 claims 57-59,61-66 ---	1-4,10, 15,16
X	WO 98 43618 A (NEORX CORP) 8 October 1998 (1998-10-08) claims 1,8-23,28-36,56-59,62-64,74-77 ---	1-3,10, 15,16
X	SOLLOTT ET AL: "Taxol inhibits neointimal smooth muscle cell accumulation after angioplasty in the rat" JOURNAL OF CLINICAL INVESTIGATION,US,NEW YORK, NY, vol. 4, no. 95, 1 April 1995 (1995-04-01), pages 1869-1876, XP002075727 ISSN: 0021-9738 page 1875, column 1, line 32-41 ---	1-4,10, 15,16
X	KOMOWSKI R ET AL: "SLOW-RELEASE TAXOL COATED GRIITM STENTS REDUCE NEOINTIMA FORMATION IN A PORCINE CORONARY IN-STENT RESTENOSIS MODEL" CIRCULATION,US,AMERICAN HEART ASSOCIATION, DALLAS, TX, vol. 96, no. 8, 1997, page 1341 XP000891206 ISSN: 0009-7322 the whole document ---	1-4,10, 15,16
X	KUNERT ET AL: "Paclitaxel inhibits development of restenosis following experimental ballon angioplasty in the rabbit carotid artery" EUROPEAN HEART JOURNAL,XX,THE EUROPEAN SOCIETY OF CARDIOLOGY, no. 17, 1996, page 368 XP002075724 ISSN: 0195-668X the whole document ---	1-3,10, 15,16
X	WO 95 03795 A (US ARMY ;KINSELLA JAMES L (US); SOLLOTT STEVEN J (US)) 9 February 1995 (1995-02-09) claims 1,7-9,12-16,20-24 ---	1-3,10, 15,16
E	WO 00 41687 A (ALVARADO ANGELICA ;QUANAM MEDICAL CORP (US); EURY ROBERT (US); FRO) 20 July 2000 (2000-07-20) the whole document --- -/--	1-4,10, 15,16

3

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inter...ional Application No
PCT/US 00/02376

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	AXEL D I ET AL: "Paclitaxel inhibits arterial smooth muscle cell proliferation and migration in vitro and in vivo using local drug delivery [see comments]." CIRCULATION, (1997 JUL 15) 96 (2) 636-45., XP000937700 the whole document ---	1-10,15, 16
Y	ZHANG, XICHEN ET AL: "Development of biodegradable polymeric paste formulations for taxol: an in vitro and in vivo study" INT. J. PHARM. (1996), 137(2), 199-208, XP000938230 the whole document ---	1-10,15, 16
P,Y	BURT, HELEN M. ET AL: "Development of copolymers of poly(DL-lactide) and methoxypolyethylene glycol as micellar carriers of paclitaxel" COLLOIDS SURF., B (1999), 16(1-4), 161-171, XP000937878 the whole document ---	1-10,15, 16
Y	ZHANG, XICHEN ET AL: "An investigation of the antitumour activity and biodistribution of polymeric micellar paclitaxel." CANCER CHEMOTHERAPY AND PHARMACOLOGY, (1997) VOL. 40, NO. 1, PP. 81-86., XP000937843 the whole document -----	1-10,15, 16

3

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 00/02376

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-9 (partial); 10, 15, 16 (complete)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: Claims 1-9, (partial); 10,15,16 (complete).

   Pharmaceutical compositions for intra pericardial administration comprising paclitaxel, in relation to the treatment of stenosis, restenosis, stent-stenosis, valvular stenosis, shunt restenosis.

2. Claims: Claims 1-9, (partial); 11(complete).

   Pharmaceutical compositions for intra pericardial administration comprising paclitaxel, in relation to the treatment of atherosclerosis.

3. Claims: Claims 1-9, (partial); 12 (complete).

   Pharmaceutical compositions for intra pericardial administration comprising paclitaxel, in relation to the treatment of transplant rejection.

4. Claims: Claims 1-9, (partial); 13 (complete).

   Pharmaceutical compositions for intra pericardial administration comprising paclitaxel, in relation to the treatment of arteritis.

5. Claims: Claims 1-9, (partial); 14 (complete).

   Pharmaceutical compositions for intra pericardial administration comprising paclitaxel, in relation to the treatment of a rheumatic condition.

6. Claims: Claims 1-9, (partial); 17 (complete).

   Pharmaceutical compositions for intra pericardial administration comprising paclitaxel, in relation to the treatment of cardiac adhesion.

7. Claims: Claims 1-9, (partial); 18 (complete).

   Pharmaceutical compositions for intra pericardial administration comprising paclitaxel, in relation to the treatment of malignant pericardial effusion.

8. Claims: Claims 1-9, (partial); 19 (complete).

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Pharmaceutical compositions for intra pericardial administration comprising paclitaxel, in relation to the treatment of cardiac rhythm disorders.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ ~~208~~ 210

Continuation of Box 3.

Although claims 1-19 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Further defect(s) under Article 17(2)(a):

Continuation of Box 3.

The wording of claim 1 "an antimicrotubule agent" relates to compounds/compositions defined by reference to a desirable characteristic or property, namely the property of interacting and adversely affecting the microtubule behaviour.
The claims cover all compounds/compositions having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for only a very limited number of such compounds/compositions. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the compounds/compositions by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search for the first invention has been carried out for those parts of the claim which appear to be clear, concise, supported and disclosed, namely for the antimicrotubule agent "paclitaxel", related compounds having the skeleton of paclitaxel, with due regard to the general idea underlying the application.
N.B. there is a mistake in the numbering of the claims: claim 7 can not depend from claim 5 and claim 8 and 9 are identical to claim 5 and 6.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Intl. ..ional Application No
PCT/US 00/02376

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 9733552	A	18-09-1997	AU	2580697 A	01-10-1997
			BR	9710646 A	11-01-2000
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CORRECTED VERSION

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(74) Agents: McMASTERS, David, D. et al.; Seed Intellectual Property Law Group PLLC, Suite 6300, 701 Fifth Avenue, Seattle, WA 98104-7092 (US).

(81) Designated States *(national)*: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

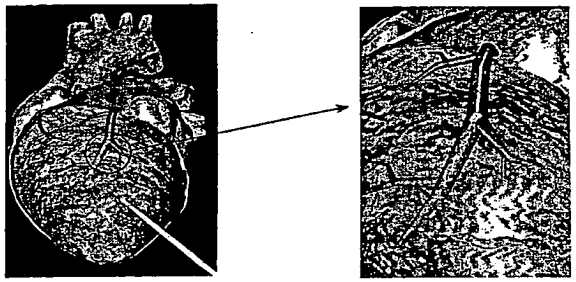
(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

[Continued on next page]

(54) Title: INTRA-PERICARDIAL DELIVERY OF ANTI-MICROTUBULE AGENTS

Intrapericardial Micellar Paclitaxel - Treatment Protocol



(57) Abstract: Methods and compositions are provided for intra-pericardial administration of anti-microtubule agents, suitable for use in treating or preventing a variety of diseases of the pericardium, heart, or, coronary vasculature.

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WO 00/44443 A3

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16 August 2001

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see PCT Gazette No. 37/2001 of 13 September 2001, Section II

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

INTRAPERICARDIAL DELIVERY OF ANTI-MICROTUBULE AGENTS

TECHNICAL FIELD

The present invention relates generally to compositions, methods and devices for administering anti-microtubule agents into the pericardium.

BACKGROUND OF THE INVENTION

According to U.S. Heart and Stroke Foundation estimates, over 60 million Americans have one or more forms of cardiovascular disease. These diseases claim approximately 1 million lives each year (41% of all deaths in the United States) and are considered the leading cause of death and disability in the developed world.

One such disease, Restenosis, is a form of chronic vascular injury leading to vessel wall thickening and loss of blood flow to the tissue supplied by the blood vessel. It occurs in response to vascular reconstructive procedures, including virtually any manipulation which attempts to relieve vessel obstructions, and is the major factor limiting the effectiveness of invasive treatments for vascular diseases. Restenosis has been a major challenge to cardiovascular research for the past 15 years.

Currently, no existing, FDA approved, treatment for the prevention of restenosis has been completely effective in humans. Systemic therapies that have been investigated include agents directed at treatment of endothelial loss, anti-platelet agents (*e.g.*, aspirin), vasodilators (*e.g.*, calcium channel blockers), antithrombotics (*e.g.*, heparin), anti-inflammatory agents (*e.g.*, steroids), agents which prevent vascular smooth muscle cell (VSMC) proliferation (*e.g.*, colchicine) and promoters of re-endothelialization (*e.g.*, vascular endothelial growth factor). Local treatments which have been investigated include local drug delivery (*e.g.*, heparin) and beta and gamma radiation. All have been disappointing in human use, primarily because they appear to act on a limited portion of the restenotic process. Systemic treatments have also encountered the additional problem of achieving adequate absorption and retention of the drug at the site of the disease to provide a lasting biological effect, without causing unfavorable systemic complications and toxicities.

Balloon angioplasty (with or without stenting) is one of the most widely used treatments for vascular disease; (other options such as laser angioplasty, however, are also

available.) While this is the treatment of choice in many cases of severe narrowing of the vasculature, about one-third of patients undergoing balloon angioplasty (source Heart and Stoke Foundation homepage) have renewed narrowing of the treated arteries (restenosis) within 6 months of the initial procedure; often serious enough to necessitate further interventions.

Vascular diseases (including for example, restenosis) are due at least in part to intimal thickening secondary to vascular smooth muscle cell (VSMC) migration, VSMC proliferation and extra-cellular matrix deposition. Briefly, vascular endothelium acts as a nonthrombogenic surface over which blood can flow smoothly and as a barrier which separates the blood components from the tissues comprising the vessel wall. Endothelial cells also release heparin sulphate, prostacyclin, EDRF and other factors that inhibit platelet and white cell adhesion, VSMC contraction, VSMC migration and VSMC proliferation. Any loss or damage to the endothelium, such as occurs during balloon angioplasty, atherectomy, or stent insertion, can result in platelet adhesion, platelet aggregation and thrombus formation. Activated platelets can release substances that produce vasoconstriction (serotonin and thromboxane) and/or promote VSMC migration and proliferation (PDGF, epidermal growth factor, TGF-□ and heparinase). Tissue factors released by the arteries stimulates clot formation resulting in a fibrin matrix into which smooth muscle cells can migrate and proliferate.

This cascade of events leads to the transformation of vascular smooth muscle cells from a contractile to a secretory phenotype. Angioplasty induced cell lysis and matrix destruction results in local release of basic fibroblast growth factor (bFGF) which in turn stimulates VSMC proliferation directly and indirectly through the induction of PDGF production. In addition to PDGF and bFGF, VSMC proliferation is also stimulated by platelet released EGF and insulin growth factor-1.

Vascular smooth muscle cells are also induced to migrate into the media and intima of the vessel. This is enabled by release and activation of matrix metalloproteases which degrade a pathway for the VSMC through the extra-cellular matrix and internal elastic lamina of the vessel wall. After migration and proliferation the vascular smooth muscle cells then deposit an extra-cellular matrix consisting of gylcosaminoglycans, elastin and collagen

which comprises the largest part of intimal thickening. A significant portion of the restenosis process may be due to remodeling of the vascular wall leading to changes in the overall size of the artery; at least some of which is secondary to proliferation within the adventitia (in addition to the media). The net result of these processes is a recurrence of the narrowing of the vascular wall which is often severe enough to require a repeat intervention.

The present invention provides compositions and methods for intrapericardially delivering an anti-microtubule agent so that disease within the pericardium, heart, or coronary vasculature (*e.g.*, restenosis, primary stenosis, or, atherosclerosis) may be treated or prevented. These compositions and methods address the problems associated with the existing procedures, offer significant advantages when compared to existing procedures, and further provides other, related advantages.

SUMMARY OF THE INVENTION

Briefly stated, the present invention provides methods for administering intrapericardially an anti-microtubule agent, suitable for treating or preventing disease of the pericardium, heart, or, coronary vasculature. The anti-microtubule agent is administered into the pericardial sac (*i.e.*, the anatomical space between the two layers of the pericardium) via a specialized needle placed through the chest wall, through the myocardium, or through the vessel wall of the vasculature supplying the heart, with or without radiographic guidance. Similarly, the agent can be placed in the pericardial cavity under direct vision during open or endoscopic surgical procedures. The anti-microtubule is present at the disease site in sufficient doses to favorably impact on cardiac conditions such as coronary stenosis, restenosis, in-stent restenosis, and atherosclerosis; surgical adhesions following cardiac surgery (open, endoscopic, or catheter –based); stenosis or failure of valve replacement surgery; accelerated atherosclerosis following cardiac transplantation; immunological rejection following cardiac transplantation (host vs. graft disease); cardiac rhythm abnormalities; rheumatic or inflammatory diseases with cardiac manifestations (*i.e.*, rheumatoid arthritis, systemic lupus erythematosis, vasculitis); infections of the myocardium or surrounding tissues; and/or primary or metastatic malignancy of the myocarium, pericardium *e.g.*, malignant pericardial effussion), or surrounding tissues. Local administration of the agent to the pericardial sac can increase the efficacy of the agent by

increasing the local dose of the agent while decreasing the systemic absorption and potential toxicity of the agent.

Representative examples of such agents include taxanes (*e.g.*, paclitaxel and docetaxel), eleutherobin, sarcodictyins, epothilones A and B, discodermolide, deuterium oxide (D_2O), hexylene glycol (2-methyl-2,4-pentanediol), tubercidin (7-deazaadenosine), LY290181 (2-amino-4-(3-pyridyl)-4H-naphtho(1,2-b)pyran-3-cardonitrile), aluminum fluoride, ethylene glycol bis-(succinimidylsuccinate), glycine ethyl ester, nocodazole, cytochalasin B, colchicine, colcemid, podophyllotoxin, benomyl, oryzalin, majusculamide C, demecolcine, methyl-2-benzimidazolecarbamate (MBC), LY195448, subtilisin, 1069C85, steganacin, combretastatin, curacin, estradiol, 2-methoxyestradiol, flavanol, rotenone, griseofulvin, vinca alkaloids, including vinblastine and vincristine, maytansinoids and ansamitocins, rhizoxin, phomopsin A, ustiloxins, dolastatin 10, dolastatin 15, halichondrins and halistatins, spongistatins, cryptophycins, rhazinilam, betaine, taurine, isethionate, HO-221, adociasulfate-2, estramustine, monoclonal anti-idiotypic antibodies, microtubule assembly promoting protein (taxol-like protein, TALP), cell swelling induced by hypotonic (190 mosmol/L) conditions, insulin (100 nmol/L) or glutamine (10 mmol/L), dynein binding, gibberelin, XCHO1 (kinesin-like protein), lysophosphatidic acid, lithium ion, plant cell wall components (*e.g.*, poly-L-lysine and extensin), glycerol buffers, Triton X-100 microtubule stabilizing buffer, microtubule associated proteins (*e.g.*, MAP2, MAP4, tau, big tau, ensconsin, elongation factor-1-alpha (EF-1α) and E-MAP-115), cellular entities (*e.g.*, histone H1, myelin basic protein and kinetochores), endogenous microtubular structures (*e.g.*, axonemal structures, plugs and GTP caps), stable tubule only polypeptide (*e.g.*, STOP145 and STOP220) and tension from mitotic forces, as well as any analogues and derivatives of any of the above. .

In certain embodiments, the anti-microtubule agent is formulated suitable to provide prolonged release of the agent at the site of administration, localize the agent to a specific site of administration, reduce the trauma and subsequent scarring associated with the procedure, or make the agent suitable for injection or surgical placement within the pericardial sac. The anti-microtubule agents may be formulated along with other compounds or compositions, such as, for example, an ointment, cream, lotion, gel, spray, foam, mousse,

coating, wrap, paste, barrier, implant, microsphere, microparticle, film or the like. Within certain embodiments, the compound or composition may function as a carrier, which may be either polymeric, or non-polymeric. Representative examples of polymeric carriers include poly(ethylene-vinyl acetate), copolymers of lactic acid and glycolic acid, poly (caprolactone), poly (lactic acid), copolymers of poly (lactic acid) and poly (caprolactone), gelatin, hyaluronic acid, collagen matrices, celluloses and albumen. Representative examples of other suitable carriers include, but are not limited to ethanol; mixtures of ethanol and glycols (*e.g.*, ethylene glycol or propylene glycol); mixtures of ethanol and isopropyl myristate or ethanol, isopropyl myristate and water (*e.g.*, 55:5:40); mixtures of ethanol and eineol or D-limonene (with or without water); glycols (*e.g.*, ethylene glycol or propylene glycol) and mixtures of glycols such as propylene glycol and water, phosphatidyl glycerol, dioleoylphosphatidyl glycerol, Transcutol®, or terpinolene; mixtures of isopropyl myristate and 1-hexyl-2-pyrrolidone, N-dodecyl-2-piperidinone or 1-hexyl-2-pyrrolidone.

These and other aspects of the present invention will become evident upon reference to the following detailed description and attached drawings. In addition, various references are set forth below which describe in more detail certain procedures, devices or compositions, and are therefore incorporated by reference in their entirety.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graphic illustration of one representative animal model which utilizes balloon injury of the LAD or LC.

Figure 2 is a bar graph which shows fracture length 28 days following balloon injury.

Figure 3 is a bar graph which shows the percentage change in diameter following balloon injury and treatment with control micelles, or, 50 mg or 10 mg paclitaxel-loaded micelles.

Figure 4 provides 2 photographs which depict swine coronary arteries following balloon injury and treatment with control micelles, or, 50 mg paclitaxel loaded micelles.

Figure 5 is a bar graph which shows neoinimal area/fracture length.

Figure 6 is a bar graph which shows the lumen diameter following balloon injury and treatment with control micelles, or, 50 mg or 10 mg paclitaxel-loaded micelles.

Figure 7 is a graph which shows the effect of IPC delivery of paclitaxel on vessel response to overstretch injury. The R^2 values for the linear regression analyses of control, HD and LD were 0.69, 0.66 and 0.44, respectively. A positive effect is presented by treated groups, which show bigger fracture length, smaller neointimal area.

Figures 8A and 8B are bar graphs which show the results of morphometric data of IPC delivery of paclitaxel after 28 days. (A) and (B) respectively depict the neointimal area and percent stenosis for each of the three groups. * indicates the values of $p < .001$ vs control.

Figures 9A and 9B are photomicrographs of two AV fistulae created in the same pig: (A) fistula treated with 5% paclitaxel EVA film and (B) not treated. Note the pronounced venous intimal thickening in the non-treated fistula (B) and the complete inhibition of intimal hyperplasia in the treated fistula (A). Also note the periadventitial fibrin layer in the treated fistula (A).

Figures 10A and 10B are high magnification photomicrographs at the anastomosis of two AV fistulae created in the same pig: (A) fistula treated with 20% paclitaxel EVA film and (B) non-treated. Note the marked venous intimal hyperplasia in the non-treated fistula (B) and the absence of intimal hyperplasia in the treated fistula (A). Also note the fibrin layer in the treated fistula.

DETAILED DESCRIPTION OF THE INVENTION

Prior to setting forth the invention, it may be helpful to an understanding thereof to set forth definitions of certain terms that will be used hereinafter.

"Anti-microtubule Agent" should be understood to include any protein, peptide, chemical, or other molecule which impairs the function of microtubules, for example, through the prevention or stabilization of polymerization. A wide variety of methods may be utilized to determine the anti-microtubule activity of a particular compound, including for example, assays described by Smith et al. (*Cancer Lett* 79(2):213-219, 1994) and Mooberry et al., (*Cancer Lett.* 96(2):261-266, 1995).

As noted above, the present invention provides methods for treating or preventing disease of the pericardium, heart, or coronary vasculature, comprising the step of administering to the pericardium, heart or, coronary vasculature an anti-microtubule agent. The anti-microtubule is presented to the disease site in sufficient doses to favorably impact on cardiac conditions such as coronary stenosis, restenosis, in-stent restenosis, and atherosclerosis; surgical adhesions following cardiac surgery (open, endoscopic, or catheter-based); stenosis or failure of valve replacement surgery; restenosis following cardiac shunt procedures; accelerated atherosclerosis following cardiac transplantation; immunological rejection following cardiac transplantation (host vs. graft disease); cardiac rhythm abnormalities; rheumatic or inflammatory diseases with cardiac manifestations (*i.e.*, rheumatoid arthritis, systemic lupus erythematosis, vasculitis); infections of the myocardium or surrounding tissues; and/or primary or metastatic malignancy of the myocarium, pericardium *e.g.*, malignant pericardial effussion), or surrounding tissues.

Briefly, a wide variety of anti-microtubule agents may be delivered, either with or without a carrier (*e.g.*, a polymer or ointment), in order to treat or prevent disease. Representative examples of such agents include taxanes (*e.g.*, paclitaxel (discussed in more detail below) and docetaxel) (Schiff et al., *Nature* 277:665-667, 1979; Long and Fairchild, *Cancer Research 54*: 4355-4361, 1994; Ringel and Horwitz, *J. Natl. Cancer Inst. 83*(4):288-291, 1991; Pazdur et al., *Cancer Treat. Rev. 19*(4):351-386, 1993), eleutherobin (*e.g.*, U.S. Patent No. 5,473,057), sarcodictyins (including sarcodictyin A), epothilones A and B (Bollag et al., *Cancer Research 55*:2325-2333, 1995), discodermolide (ter Haar et al., *Biochemistry 35*:243-250, 1996), deuterium oxide (D_2O) (James and Lefebvre, *Genetics 130*(2):305-314, 1992; Sollott et al., *J. Clin. Invest. 95*:1869-1876, 1995), hexylene glycol (2-methyl-2,4-pentanediol) (Oka et al., *Cell Struct. Funct. 16*(2):125-134, 1991), tubercidin (7-deazaadenosine) (Mooberry et al., *Cancer Lett. 96*(2):261-266, 1995), LY290181 (2-amino-4-(3-pyridyl)-4H-naphtho(1,2-b)pyran-3-cardonitrile) (Panda et al., *J. Biol. Chem. 272*(12):7681-7687, 1997; Wood et al., *Mol. Pharmacol. 52*(3):437-444, 1997), aluminum fluoride (Song et al., *J. Cell. Sci. Suppl. 14*:147-150, 1991), ethylene glycol bis-(succinimidylsuccinate) (Caplow and Shanks, *J. Biol. Chem. 265*(15):8935-8941, 1990), glycine ethyl ester (Mejillano et al., *Biochemistry 31*(13):3478-3483, 1992), nocodazole

(Ding et al., *J. Exp. Med. 171*(3):715-727, 1990; Dotti et al., *J. Cell Sci. Suppl. 15*:75-84, 1991; Oka et al., *Cell Struct. Funct. 16*(2):125-134, 1991; Weimer et al., *J. Cell. Biol. 136*(1):71-80, 1997), cytochalasin B (Illinger et al., *Biol. Cell 73*(2-3):131-138, 1991), colchicine and CI 980 (Allen et al., *Am. J. Physiol. 261*(4 Pt. 1):L315-L321, 1991; Ding et al., *J. Exp. Med. 171*(3): 715-727, 1990; Gonzalez et al., *Exp. Cell. Res. 192*(1):10-15, 1991; Stargell et al., *Mol. Cell. Biol. 12*(4):1443-1450, 1992; Garcia et al., *Antican. Drugs 6*(4):533-544, 1995), colcemid (Barlow et al., *Cell. Motil. Cytoskeleton 19*(1):9-17, 1991; Meschini et al., *J. Microsc. 176*(Pt. 3): 204-210, 1994; Oka et al., *Cell Struct. Funct. 16*(2):125-134, 1991), podophyllotoxin (Ding et al., *J. Exp. Med. 171*(3):715-727, 1990), benomyl (Hardwick et al., *J. Cell. Biol. 131*(3):709-720, 1995; Shero et al., *Genes Dev.* 5(4): 549-560, 1991), oryzalin (Stargell et al., *Mol. Cell. Biol.* 12(4): 1443-1450, 1992), majusculamide C (Moore, *J. Ind. Microbiol.* 16(2): 134-143, 1996), demecolcine (Van Dolah and Ramsdell, *J. Cell. Physiol.* 166(1): 49-56, 1996; Wiemer et al., *J. Cell. Biol.* 136(1): 71-80, 1997), methyl-2-benzimidazolecarbamate (MBC) (Brown et al., *J. Cell. Biol.* 123(2): 387-403, 1993), LY195448 (Barlow & Cabral, *Cell Motil. Cytoskel.* 19: 9-17, 1991), subtilisin (Saoudi et al., *J. Cell Sci.* 108: 357-367, 1995), 1069C85 (Raynaud et al., *Cancer Chemother. Pharmacol.* 35: 169-173, 1994), steganacin (Hamel, *Med. Res. Rev. 16*(2):207-231, 1996), combretastatins (Hamel, *Med. Res. Rev.* 16(2):207-231, 1996), curacins (Hamel, *Med. Res. Rev.* 16(2): 207-231, 1996), estradiol (Aizu-Yokata et al., *Carcinogen.* 15(9):1875-1879, 1994), 2-methoxyestradiol (Hamel, *Med. Res. Rev.* 16(2):207-231, 1996), flavanols (Hamel, *Med. Res. Rev.* 16(2):207-231, 1996), rotenone (Hamel, *Med. Res. Rev.* 16(2):207-231, 1996), griseofulvin (Hamel, *Med. Res. Rev.* 16(2):207-231, 1996), vinca alkaloids, including vinblastine and vincristine (Ding et al., *J. Exp. Med.* 171(3):715-727, 1990; Dirk et al., *Neurochem. Res.* 15(11):1135-1139, 1990; Hamel, *Med. Res. Rev.* 16(2):207-231, 1996; Illinger et al., *Biol. Cell* 73(2-3):131-138, 1991; Wiemer et al., *J. Cell. Biol.* 136(1):71-80, 1997), maytansinoids and ansamitocins (Hamel, *Med. Res. Rev.* 16(2):207-231, 1996), rhizoxin (Hamel, *Med. Res. Rev.* 16(2):207-231, 1996), phomopsin A (Hamel, *Med. Res. Rev.* 16(2):207-231, 1996), ustiloxins (Hamel, *Med. Res. Rev.* 16(2):207-231, 1996), dolastatin 10 (Hamel, *Med. Res. Rev.* 16(2):207-231, 1996), dolastatin 15 (Hamel, *Med. Res. Rev.* 16(2):207-231, 1996), halichondrins and halistatins (Hamel, *Med. Res. Rev.* 16(2):207-231,

1996), spongistatins (Hamel, *Med. Res. Rev.* 16(2):207-231, 1996), cryptophycins (Hamel, *Med. Res. Rev.* 16(2):207-231, 1996), rhazinilam (Hamel, *Med. Res. Rev.* 16(2):207-231, 1996), betaine (Hashimoto et al., *Zool. Sci.* 1:195-204, 1984), taurine (Hashimoto et al., *Zool. Sci.* 1:195-204, 1984), isethionate (Hashimoto et al., *Zool. Sci.* 1:195-204, 1984), HO-221 (Ando et al., *Cancer Chemother. Pharmacol.* 37:63-69, 1995), adociasulfate-2 (Sakowicz et al., *Science* 280:292-295, 1998), estramustine (Panda et al., *Proc. Natl. Acad. Sci. USA* 94:10560-10564, 1997), monoclonal anti-idiotypic antibodies (Leu et al., *Proc. Natl. Acad. Sci. USA* 91(22):10690-10694, 1994), microtubule assembly promoting protein (taxol-like protein, TALP) (Hwang et al., *Biochem. Biophys. Res. Commun.* 208(3):1174-1180, 1995), cell swelling induced by hypotonic (190 mosmol/L) conditions, insulin (100 nmol/L) or glutamine (10 mmol/L) (Haussinger et al., *Biochem. Cell. Biol.* 72(1-2):12-19, 1994), dynein binding (Ohba et al., *Biochim. Biophys. Acta* 1158(3):323-332, 1993), gibberelin (Mita and Shibaoka, *Protoplasma* 119(1/2):100-109, 1984), XCHO1 (kinesin-like protein) (Yonetani et al., *Mol. Biol. Cell* 7(suppl):211A, 1996), lysophosphatidic acid (Cook et al., *Mol. Biol. Cell* 6(suppl):260A, 1995), lithium ion (Bhattacharyya and Wolff, *Biochem. Biophys. Res. Commun.* 73(2):383-390, 1976), plant cell wall components (*e.g.*, poly-L-lysine and extensin) (Akashi et al., *Planta* 182(3):363-369, 1990), glycerol buffers (Schilstra et al., *Biochem. J.* 277(Pt. 3):839-847, 1991; Farrell and Keates, *Biochem. Cell. Biol.* 68(11): 1256-1261, 1990; Lopez et al., *J. Cell. Biochem.* 43(3): 281-291, 1990), Triton X-100 microtubule stabilizing buffer (Brown et al., *J. Cell Sci.* 104(Pt. 2): 339-352, 1993; Safiejko-Mroczka and Bell, *J. Histochem. Cytochem.* 44(6): 641-656, 1996), microtubule associated proteins (*e.g.*, MAP2, MAP4, tau, big tau, ensconsin, elongation factor-1-alpha (EF-1α) and E-MAP-115) (Burgess et al., *Cell Motil. Cytoskeleton* 20(4): 289-300, 1991; Saoudi et al., *J. Cell. Sci.* 108(Pt. 1): 357-367, 1995; Bulinski and Bossler, *J. Cell. Sci.* 107(Pt. 10): 2839-2849, 1994; Ookata et al., *J. Cell Biol.* 128(5): 849-862, 1995; Boyne et al., *J. Comp. Neurol.* 358(2): 279-293, 1995; Ferreira and Caceres, *J. Neurosci.* 11(2): 392-400, 1991; Thurston et al., *Chromosoma* 105(1): 20-30, 1996; Wang et al., *Brain Res. Mol. Brain Res.* 38(2): 200-208, 1996; Moore and Cyr, *Mol. Biol. Cell* 7(suppl): 221-A, 1996; Masson and Kreis, *J. Cell Biol.* 123(2), 357-371, 1993), cellular entities (*e.g.*, histone H1, myelin basic protein and kinetochores) (Saoudi et al., *J. Cell. Sci.* 108(Pt. 1): 357-367, 1995; Simerly et al., *J. Cell Biol.* 111(4): 1491-1504,

1990), endogenous microtubular structures (*e.g.*, axonemal structures, plugs and GTP caps) (Dye et al., *Cell Motil. Cytoskeleton* 21(3): 171-186, 1992; Azhar and Murphy, *Cell Motil. Cytoskeleton* 15(3): 156-161, 1990; Walker et al., *J. Cell Biol.* 114(1): 73-81, 1991; Drechsel and Kirschner, *Curr. Biol.* 4(12): 1053-1061, 1994), stable tubule only polypeptide (*e.g.*, STOP145 and STOP220) (Pirollet et al., *Biochim. Biophys. Acta* 1160(1): 113-119, 1992; Pirollet et al., *Biochemistry* 31(37): 8849-8855, 1992; Bosc et al., *Proc. Natl. Acad. Sci. USA* 93(5): 2125-2130, 1996; Margolis et al., *EMBO J.* 9(12): 4095-4102, 1990) and tension from mitotic forces (Nicklas and Ward, *J. Cell Biol.* 126(5): 1241-1253, 1994), as well as any analogues and derivatives of any of the above. Such compounds can act by either depolymerizing microtubules (*e.g.*, colchicine and vinblastine), or by stabilizing microtubule formation (*e.g.*, paclitaxel).

Within one preferred embodiment of the invention, the therapeutic agent is paclitaxel, a compound which disrupts microtubule formation by binding to tubulin to form abnormal mitotic spindles. Briefly, paclitaxel is a highly derivatized diterpenoid (Wani et al., *J. Am. Chem. Soc. 93*:2325, 1971) which has been obtained from the harvested and dried bark of *Taxus brevifolia* (Pacific Yew) and *Taxomyces Andreanae* and *Endophytic Fungus* of the Pacific Yew (Stierle et al., *Science 60*:214-216, 1993). "Paclitaxel" (which should be understood herein to include prodrugs, analogues and derivatives such as, for example, TAXOL®, TAXOTERE®, Docetaxel, 10-desacetyl analogues of paclitaxel and 3'N-desbenzoyl-3'N-t-butoxy carbonyl analogues of paclitaxel) may be readily prepared utilizing techniques known to those skilled in the art (*see e.g.*, Schiff et al., *Nature 277*:665-667, 1979; Long and Fairchild, *Cancer Research 54*:4355-4361, 1994; Ringel and Horwitz, *J. Natl. Cancer Inst. 83*(4):288-291, 1991; Pazdur et al., *Cancer Treat. Rev. 19*(4):351-386, 1993; WO 94/07882; WO 94/07881; WO 94/07880; WO 94/07876; WO 93/23555; WO 93/10076; WO94/00156; WO 93/24476; EP 590267; WO 94/20089; U.S. Patent Nos. 5,294,637; 5,283,253; 5,279,949; 5,274,137; 5,202,448; 5,200,534; 5,229,529; 5,254,580; 5,412,092; 5,395,850; 5,380,751; 5,350,866; 4,857,653; 5,272,171; 5,411,984; 5,248,796; 5,248,796; 5,422,364; 5,300,638; 5,294,637; 5,362,831; 5,440,056; 4,814,470; 5,278,324; 5,352,805; 5,411,984; 5,059,699; 4,942,184; *Tetrahedron Letters 35*(52):9709-9712, 1994; *J. Med. Chem. 35*:4230-4237, 1992; *J. Med. Chem. 34*:992-998, 1991; *J. Natural Prod. 57*(10):1404-

1410, 1994; *J. Natural Prod. 57*(11):1580-1583, 1994; *J. Am. Chem. Soc. 110*:6558-6560, 1988), or obtained from a variety of commercial sources, including for example, Sigma Chemical Co., St. Louis, Missouri (T7402 - from *Taxus brevifolia*).

Representative examples of such paclitaxel derivatives or analogues include 7-deoxy-docetaxol, 7,8-cyclopropataxanes, N-substituted 2-azetidones, 6,7-epoxy paclitaxels, 6,7-modified paclitaxels, 10-desacetoxytaxol, 10-deacetyltaxol (from 10-deacetylbaccatin III), phosphonooxy and carbonate derivatives of taxol, taxol 2',7-di(sodium 1,2-benzenedicarboxylate, 10-desacetoxy-11,12-dihydrotaxol-10,12(18)-diene derivatives, 10-desacetoxytaxol, Protaxol (2'-and/or 7-O-ester derivatives), (2'-and/or 7-O-carbonate derivatives), asymmetric synthesis of taxol side chain, fluoro taxols, 9-deoxotaxane, (13-acetyl-9-deoxobaccatine III, 9-deoxotaxol, 7-deoxy-9-deoxotaxol, 10-desacetoxy-7-deoxy-9-deoxotaxol, Derivatives containing hydrogen or acetyl group and a hydroxy and tert-butoxycarbonylamino, sulfonated 2'-acryloyltaxol and sulfonated 2'-O-acyl acid taxol derivatives, succinyltaxol, 2'-γ-aminobutyryltaxol formate, 2'-acetyl taxol, 7-acetyl taxol, 7-glycine carbamate taxol, 2'-OH-7-PEG(5000) carbamate taxol, 2'-benzoyl and 2',7-dibenzoyl taxol derivatives, other prodrugs (2'-acetyltaxol; 2',7-diacetyltaxol; 2'succinyltaxol; 2'-(beta-alanyl)-taxol); 2'gamma-aminobutyryltaxol formate; ethylene glycol derivatives of 2'-succinyltaxol; 2'-glutaryltaxol; 2'-(N,N-dimethylglycyl) taxol; 2'-(2-(N,N-dimethylamino)propionyl)taxol; 2'orthocarboxybenzoyl taxol; 2'aliphatic carboxylic acid derivatives of taxol, Prodrugs {2'(N,N-diethylaminopropionyl)taxol, 2'(N,N-dimethylglycyl)taxol, 7(N,N-dimethylglycyl)taxol, 2',7-di-(N,N-dimethylglycyl)taxol, 7(N,N-diethylaminopropionyl)taxol, 2',7-di(N,N-diethylaminopropionyl)taxol, 2'-(L-glycyl)taxol, 7-(L-glycyl)taxol, 2',7-di(L-glycyl)taxol, 2'-(L-alanyl)taxol, 7-(L-alanyl)taxol, 2',7-di(L-alanyl)taxol, 2'-(L-leucyl)taxol, 7-(L-leucyl)taxol, 2',7-di(L-leucyl)taxol, 2'-(L-isoleucyl)taxol, 7-(L-isoleucyl)taxol, 2',7-di(L-isoleucyl)taxol, 2'-(L-valyl)taxol, 7-(L-valyl)taxol, 2'7-di(L-valyl)taxol, 2'-(L-phenylalanyl)taxol, 7-(L-phenylalanyl)taxol, 2',7-di(L-phenylalanyl)taxol, 2'-(L-prolyl)taxol, 7-(L-prolyl)taxol, 2',7-di(L-prolyl)taxol, 2'-(L-lysyl)taxol, 7-(L-lysyl)taxol, 2',7-di(L-lysyl)taxol; 2'-(L-glutamyl)taxol, 7-(L-glutamyl)taxol, 2',7-di(L-glutamyl)taxol, 2'-(L-arginyl)taxol, 7-(L-arginyl)taxol, 2',7-di(L-arginyl)taxol}, Taxol analogs with modified phenylisoserine side chains, taxotere, (N-debenzoyl-N-tert-

(butoxycaronyl)-10-deacetyltaxol, and taxanes (*e.g.*, baccatin III, cephalomannine, 10-deacetylbaccatin III, brevifoliol, yunantaxusin and taxusin).

FORMULATIONS

As noted above, therapeutic anti-microtubule agents described herein may be formulated in a variety of manners, and thus may additionally comprise a carrier. In this regard, a wide variety of carriers may be selected of either polymeric or non-polymeric origin.

For example, within one embodiment of the invention a wide variety of polymeric carriers may be utilized to contain and/or deliver one or more of the therapeutic agents discussed above, including for example both biodegradable and non-biodegradable compositions. Representative examples of biodegradable compositions include albumin, collagen, gelatin, hyaluronic acid, starch, cellulose (methylcellulose, hydroxypropylcellulose, hydroxypropylmethylcellulose,, hydroxyethylcellulose, carboxymethylcellulose, cellulose acetate phthalate, cellulose acetate succinate, hydroxypropylmethylcellulose phthalate), casein, dextrans, polysaccharides, fibrinogen, poly(D,L lactide), poly(D,L-lactide-co-glycolide), poly(glycolide), poly(hydroxybutyrate), poly(alkylcarbonate) and poly(orthoesters), polyesters, poly(hydroxyvaleric acid), polydioxanone, poly(ethylene terephthalate), poly(malic acid), poly(tartronic acid), polyanhydrides, polyphosphazenes, poly(amino acids) and their copolymers (*see generally*, Illum, L., Davids, S.S. (eds.) "Polymers in Controlled Drug Delivery" Wright, Bristol, 1987; Arshady, *J. Controlled Release 17*:1-22, 1991; Pitt, *Int. J. Phar. 59*:173-196, 1990; Holland et al., *J. Controlled Release 4*:155-0180, 1986). Representative examples of nondegradable polymers include poly(ethylene-vinyl acetate) ("EVA") copolymers, silicone rubber, acrylic polymers (polyacrylic acid, polymethylacrylic acid, polymethylmethacrylate, polyalkylcynoacrylate), polyethylene, polyproplene, polyamides (nylon 6,6), polyurethane, poly(ester urethanes), poly(ether urethanes), poly(ester-urea), polyethers (poly(ethylene oxide), poly(propylene oxide), Pluronics and poly(tetramethylene glycol)), silicone rubbers and vinyl polymers (polyvinylpyrrolidone, poly(vinyl alcohol), poly(vinyl acetate phthalate). Polymers may also be developed which are either anionic (*e.g.*, alginate, carrageenin, carboxymethyl cellulose and poly(acrylic acid), or cationic (*e.g.*, chitosan, poly-L-lysine, polyethylenimine, and poly (allyl amine)) (*see generally*, Dunn et al., *J. Applied Polymer Sci. 50*:353-365, 1993; Cascone

et al., *J. Materials Sci.: Materials in Medicine 5*:770-774, 1994; Shiraishi et al., *Biol. Pharm. Bull. 16*(11):1164-1168, 1993; Thacharodi and Rao, *Int'l J. Pharm. 120*:115-118, 1995; Miyazaki et al., *Int'l J. Pharm. 118*:257-263, 1995). Particularly preferred polymeric carriers include poly(ethylene-vinyl acetate), poly (D,L-lactic acid) oligomers and polymers, poly (L-lactic acid) oligomers and polymers, poly (glycolic acid), copolymers of lactic acid and glycolic acid, poly (caprolactone), poly (valerolactone), polyanhydrides, copolymers of poly (caprolactone) or poly (lactic acid) with a polyethylene glycol (*e.g.*, MePEG), and blends thereof.

Polymeric carriers can be fashioned in a variety of forms, with desired release characteristics and/or with specific desired properties. For example, polymeric carriers may be fashioned to release a therapeutic agent upon exposure to a specific triggering event such as pH (*see e.g.*, Heller et al., "Chemically Self-Regulated Drug Delivery Systems," in *Polymers in Medicine III*, Elsevier Science Publishers B.V., Amsterdam, 1988, pp. 175-188; Kang et al., *J. Applied Polymer Sci. 48*:343-354, 1993; Dong et al., *J. Controlled Release 19:171-178, 1992;* Dong and Hoffman, *J. Controlled Release 15*:141-152, 1991; Kim et al., *J. Controlled Release 28*:143-152, 1994; Cornejo-Bravo et al., *J. Controlled Release 33*:223-229, 1995; Wu and Lee, *Pharm. Res. 10*(10):1544-1547, 1993; Serres et al., *Pharm. Res. 13*(2):196-201, 1996; Peppas, "Fundamentals of pH- and Temperature-Sensitive Delivery Systems," in Gurny et al. (eds.), *Pulsatile Drug Delivery*, Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart, 1993, pp. 41-55; Doelker, "Cellulose Derivatives," 1993, in Peppas and Langer (eds.), *Biopolymers I*, Springer-Verlag, Berlin). Representative examples of pH-sensitive polymers include poly(acrylic acid) and its derivatives (including for example, homopolymers such as poly(aminocarboxylic acid); poly(acrylic acid); poly(methyl acrylic acid), copolymers of such homopolymers, and copolymers of poly(acrylic acid) and acrylmonomers such as those discussed above. Other pH sensitive polymers include polysaccharides such as cellulose acetate phthalate; hydroxypropylmethylcellulose phthalate; hydroxypropylmethylcellulose acetate succinate; cellulose acetate trimellilate; and chitosan. Yet other pH sensitive polymers include any mixture of a pH sensitive polymer and a water soluble polymer.

Likewise, polymeric carriers can be fashioned which are temperature sensitive (*see e.g.*, Chen et al., "Novel Hydrogels of a Temperature-Sensitive Pluronic Grafted to a Bioadhesive Polyacrylic Acid Backbone for Vaginal Drug Delivery," in *Proceed. Intern. Symp. Control. Rel. Bioact. Mater. 22*:167-168, Controlled Release Society, Inc., 1995; Okano, "Molecular Design of Stimuli-Responsive Hydrogels for Temporal Controlled Drug Delivery," in *Proceed. Intern. Symp. Control. Rel. Bioact. Mater. 22*:111-112, Controlled Release Society, Inc., 1995; Johnston et al., *Pharm. Res. 9*(3):425-433, 1992; Tung, *Int'l J. Pharm. 107*:85-90, 1994; Harsh and Gehrke, *J. Controlled Release 17*:175-186, 1991; Bae et al., *Pharm. Res. 8*(4):531-537, 1991; Dinarvand and D'Emanuele, *J. Controlled Release 36*:221-227, 1995; Yu and Grainger, "Novel Thermo-sensitive Amphiphilic Gels: Poly N-isopropylacrylamide-co-sodium acrylate-co-n-N-alkylacrylamide Network Synthesis and Physicochemical Characterization," Dept. of Chemical & Biological Sci., Oregon Graduate Institute of Science & Technology, Beaverton, OR, pp. 820-821; Zhou and Smid, "Physical Hydrogels of Associative Star Polymers," Polymer Research Institute, Dept. of Chemistry, College of Environmental Science and Forestry, State Univ. of New York, Syracuse, NY, pp. 822-823; Hoffman et al., "Characterizing Pore Sizes and Water 'Structure' in Stimuli-Responsive Hydrogels," Center for Bioengineering, Univ. of Washington, Seattle, WA, p. 828; Yu and Grainger, "Thermo-sensitive Swelling Behavior in Crosslinked N-isopropylacrylamide Networks: Cationic, Anionic and Ampholytic Hydrogels," Dept. of Chemical & Biological Sci., Oregon Graduate Institute of Science & Technology, Beaverton, OR, pp. 829-830; Kim et al., *Pharm. Res. 9*(3):283-290, 1992; Bae et al., *Pharm. Res. 8*(5):624-628, 1991; Kono et al., *J. Controlled Release 30*:69-75, 1994; Yoshida et al., *J. Controlled Release 32*:97-102, 1994; Okano et al., *J. Controlled Release 36*:125-133, 1995; Chun and Kim, *J. Controlled Release 38*:39-47, 1996; D'Emanuele and Dinarvand, *Int'l J. Pharm. 118*:237-242, 1995; Katono et al., *J. Controlled Release 16*:215-228, 1991; Hoffman, "Thermally Reversible Hydrogels Containing Biologically Active Species," in Migliaresi et al. (eds.), *Polymers in Medicine III*, Elsevier Science Publishers B.V., Amsterdam, 1988, pp. 161-167; Hoffman, "Applications of Thermally Reversible Polymers and Hydrogels in Therapeutics and Diagnostics," in *Third International Symposium on Recent Advances in Drug Delivery Systems*, Salt Lake City, UT, Feb. 24-27, 1987, pp. 297-305; Gutowska et al.,

J. Controlled Release 22:95-104, 1992; Palasis and Gehrke, *J. Controlled Release 18*:1-12, 1992; Paavola et al., *Pharm. Res. 12*(12):1997-2002, 1995).

Representative examples of thermogelling polymers, and their gelatin temperature (LCST (°C)) include homopolymers such as poly(N-methyl-N-n-propylacrylamide), 19.8; poly(N-n-propylacrylamide), 21.5; poly(N-methyl-N-isopropylacrylamide), 22.3; poly(N-n-propylmethacrylamide), 28.0; poly(N-isopropylacrylamide), 30.9; poly(N, n-diethylacrylamide), 32.0; poly(N-isopropylmethacrylamide), 44.0; poly(N-cyclopropylacrylamide), 45.5; poly(N-ethylmethyacrylamide), 50.0; poly(N-methyl-N-ethylacrylamide), 56.0; poly(N-cyclopropylmethacrylamide), 59.0; poly(N-ethylacrylamide), 72.0. Moreover thermogelling polymers may be made by preparing copolymers between (among) monomers of the above, or by combining such homopolymers with other water soluble polymers such as acrylmonomers (*e.g.*, acrylic acid and derivatives thereof such as methylacrylic acid, acrylate and derivatives thereof such as butyl methacrylate, acrylamide, and N-n-butyl acrylamide).

Other representative examples of thermogelling polymers include cellulose ether derivatives such as hydroxypropyl cellulose, 41°C; methyl cellulose, 55°C; hydroxypropylmethyl cellulose, 66°C; and ethylhydroxyethyl cellulose, and Pluronics such as F-127, 10 - 15°C; L-122, 19°C; L-92, 26°C; L-81, 20°C; and L-61, 24°C.

A wide variety of forms may be fashioned by the polymeric carriers of the present invention, including for example, rod-shaped devices, pellets, slabs, or capsules (*see e.g.*, Goodell et al., *Am. J. Hosp. Pharm. 43*:1454-1461, 1986; Langer et al., "Controlled release of macromolecules from polymers", in *Biomedical Polymers, Polymeric Materials and Pharmaceuticals for Biomedical Use*, Goldberg, E.P., Nakagim, A. (eds.) Academic Press, pp. 113-137, 1980; Rhine et al., *J. Pharm. Sci. 69*:265-270, 1980; Brown et al., *J. Pharm. Sci. 72*:1181-1185, 1983; and Bawa et al., *J. Controlled Release 1*:259-267, 1985). Therapeutic agents may be linked by occlusion in the matrices of the polymer, bound by covalent linkages, or encapsulated in microcapsules. Within certain preferred embodiments of the invention, therapeutic compositions are provided in non-capsular formulations such as microspheres (ranging from nanometers to micrometers in size), pastes, threads of various size, films and sprays.

Preferably, therapeutic compositions of the present invention are fashioned in a manner appropriate to the intended use. Within certain aspects of the present invention, the therapeutic composition should be biocompatible, and release one or more therapeutic agents over a period of several days to months. For example, "quick release" or "burst" therapeutic compositions are provided that release greater than 10%, 20%, or 25% (w/v) of a therapeutic agent (*e.g.*, paclitaxel) over a period of 7 to 10 days. Such "quick release" compositions should, within certain embodiments, be capable of releasing chemotherapeutic levels (where applicable) of a desired agent. Within other embodiments, "low release" therapeutic compositions are provided that release less than 1% (w/v) of a therapeutic agent over a period of 7 to 10 days. Further, therapeutic compositions of the present invention should preferably be stable for several months and capable of being produced and maintained under sterile conditions.

Within certain aspects of the present invention, therapeutic compositions may be fashioned in any size ranging from 50 nm to 500 μm, depending upon the particular use. Alternatively, such compositions may also be readily applied as a "spray", which solidifies into a film or coating. Such sprays may be prepared from microspheres of a wide array of sizes, including for example, from 0.1 μm to 3 μm, from 10 μm to 30 μm, and from 30 μm to 100 μm.

Therapeutic compositions of the present invention may also be prepared in a variety of "paste" or gel forms. For example, within one embodiment of the invention, therapeutic compositions are provided which are liquid at one temperature (*e.g.*, temperature greater than 37°C, such as 40°C, 45°C, 50°C, 55°C or 60°C), and solid or semi-solid at another temperature (*e.g.*, ambient body temperature, or any temperature lower than 37°C). Such "thermopastes" may be readily made given the disclosure provided herein.

FORMULATION AND ADMINISTRATION

As noted above, anti-microtubule agents of the present invention may be formulated in a variety of forms suitable for administration. Further, the compositions of the present invention may be formulated to contain more than one anti-microtubule agents, to contain a variety of additional compounds, to have certain physical properties (*e.g.*, elasticity, a particular melting point, or a specified release rate). Within certain embodiments of the

invention, compositions may be combined in order to achieve a desired effect (*e.g.*, several preparations of microspheres may be combined in order to achieve both a quick and a slow or prolonged release of one or more anti-microtubule agents).

Anti-microtubule agents may be administered either alone, or in combination with pharmaceutically or physiologically acceptable carrier, excipients or diluents. Generally, such carriers should be nontoxic to recipients at the dosages and concentrations employed. Ordinarily, the preparation of such compositions entails combining the therapeutic agent with buffers, antioxidants such as ascorbic acid, low molecular weight (less than about 10 residues) polypeptides, proteins, amino acids, carbohydrates including glucose, sucrose or dextrins, chelating agents such as EDTA, glutathione and other stabilizers and excipients. Neutral buffered saline or saline mixed with nonspecific serum albumin are exemplary appropriate diluents.

CLINICAL ADMINISTRATION

The anti-microtubule agent or composition is administered into the pericardial sac (*i.e.*, the anatomical space between the two layers of the pericardium) by, for example, direct injection via specialized needle placed through the chest wall, through the myocardium, or through the vessel wall of the vasculature supplying the heart, with or without radiographic guidance. Similarly, the agent can be placed in the pericardial cavity under direct vision during open or endoscopic surgical procedures.

With direct injection, a catheter is placed in the pericardial sac preferably utilizing ultrasound, CT, fluoroscopic, MRI or endoscopic guidance (See *e.g.*, U.S. Patent Nos. 5,840,059 and 5,797,870). Within certain embodiments, specialized percutaneous injection devices are used to safely deliver the anti-microtubule agent into the pericardial sac via the thoracic wall (*e.g.*, with a Saphenous Vein Harvester such as GSI's ENDOsaph, or Comedicus Inc.'s PerDUCER (Pericardial Access Device)). In another embodiment, the antimicrotubule agent or composition is injected into the pericardium via a catheter (or other specialized injection device) placed trans-myocardially through the right or left ventricle. Similarly, the antimicrotubule agent or composition (*e.g.*, paclitaxel and a polymer) may be administered trans-myocardially through the right atrium. (See, *e.g.*, U.S. Patent Nos. 5,797,870 and 5,269,326). Briefly, the right atrium lies between the pericardium and the

epicardium. An appropriate catheter is guided into the right atrium and positioned parallel with the wall of the pericardium. This positioning allows piercing of the right atrium (either by the catheter, or by an instrument that is passed within the catheter), without risk of damage to either the pericardium or the epicardium. The catheter can then be passed into the pericardial space, or an instrument passed through the lumen of the catheter into the pericardial space. The anti-microtubule agent is then administered via the catheter into the pericardial space.

In open procedures, access to the pericardium, heart, or coronary vasculature is gained operatively, by, for example, sub-xiphoid entry, a thoracotomy, open heart surgery, or endoscopic procedures. Preferably, the thoracotomy should be minimal, through an intercostal space for example. Fluoroscopy, or ultrasonic visualization may be utilized to assist in any of these procedures. The anti-microtubule agent is then administered directly to the required site on the heart surface (*e.g.*, the coronary arteries) as a paste, gel, wrap or solution. This placement may be accomplished by the surgeon directly (open surgery) or via a delivery port in a endoscopic device (endoscopic surgery).

The anti-microtubule agent is administered in a dosage and formulation which results in clinical improvement of the patient. As mentioned previously, the compositions and methods described herein are suitable for the treatment of a variety of diseases of the heart and surrounding tissues, including, but not limited to: coronary stenosis, restenosis, in-stent restenosis, and atherosclerosis; surgical adhesions following cardiac surgery (open, endoscopic, or catheter –based); stenosis or failure of valve replacement surgery; restenosis following cardiac shunt procedures; accelerated atherosclerosis following cardiac transplantation; immunological rejection following cardiac transplantation (host vs. graft disease); cardiac rhythm abnormalities; rheumatic or inflammatory diseases with cardiac manifestations (*i.e.*, rheumatoid arthritis, systemic lupus erythematosis, vasculitis); infections of the myocardium or surrounding tissues; and/or primary or metastatic malignancy of the myocardium, pericardium (*e.g.*, malignant pericardial effussion), or surrounding tissues.

For the embodiments described below, an antimicrotubule agent such as paclitaxel is administered into the pericardium at a dosage ranging from 100 ug to 50 mg, depending on the mode of administration and the type of carrier, if any, for delivery. It

should be apparent to one of skill in the art that any method of gaining access to the pericardial sac would be acceptable for the purposes of this invention. It will also be apparent to one of skill in the art that other anti-microtubule agents would be acceptable for the purposes of this invention if given at biologically equivalent doses to those described for paclitaxel.

TREATMENT OF STENOSIS, RESTENOSIS, IN-STENT RESTENOSIS

For treatment of coronary stenosis, restenosis, or in-stent restenosis, a single direct injection of an anti-microtubule into the pericardial sac is the preferred intervention. This injection is administered before, during or after a vascular intervention such as balloon angioplasty (PTCA), stenting, atherectomy, laser ablation, rotary ablation, or surgical bypass (including open and endoscopic procedures). In a preferred embodiment, the anti-microtubule agent is administered as an aqueous solution or in a sustained-release form (*i.e.*, in association with a polymeric or nonpolymeric carrier) into the pericardial sac using the methods described previously. In a particularly preferred embodiment, paclitaxel (or an analogue or derivative thereof) is administered as a solution (*e.g.*, micellar paclitaxel) or in a sustained-release preparation (*e.g.*, PLA microspheres or hyaluronic acid microspheres containing paclitaxel) at a dose of 1 to 50 mg/m^2 which disperses throughout the pericardial space. The sustained-release preparation is designed to release therapeutic levels of paclitaxel over a period of several days to several months, depending on the indication.

For certain clinical indications (*e.g.*, the treatment of instent restenosis, restenosis following cardiac bypass surgery), the anti-microtubule agent may also be administered directly to the surface of the stented (or bypassed) coronary artery (or saphenous vein graft) to prevent closure of the stented vessel. If the drug is administered under direct vision (open surgical or endoscopic procedures), then an anti-microtubule agent contained in a paste or gel formulation (*e.g.*, paclitaxel contained in hyaluronic acid, in fibrin, or in poly-lactic acid) is the preferred embodiment. Here the physician is able to directly apply the anti-microtubule agent directly to the outer (adventitial) surface of the diseased coronary artery or tissue via the pericardial sac. Paclitaxel at a total dose of 1 to 75 mg/m^2 delivered over a period of 24 hours to 6 months in an injectable gel or paste is a particularly effective embodiment. Alternatively the anti-microtubule agent can be injected into the wall of the

coronary artery via needles/catheters suitable for this purpose. Although a single injection is preferred, when required, repeated injections can be performed to deliver the anti-microtubule agent to the pericardial space over longer periods of time.

Regardless of the methods employed, the anti-microtubule agent should be capable of reducing the signs and/or symptoms of coronary artery obstruction such as chest pain (angina), syncope, dyspnea, orthopnea, radiating pain (shoulder, arm pain), nausea, or diaphoresis. The treatment may also preserve luminal area following vascular intervention, reduce the incidence of total occlusion (myocardial infarction), prolong the effectiveness of PTCA or stenting, or preserve myocardial perfusion as demonstrated by standard cardiac function and radiographic methods.

TREATMENT OF ATHEROSCLEROSIS

For the treatment of progressive atherosclerosis, an anti-microtubule agent is injected periodically (*e.g.*, once every few months) directly into the pericardial sac utilizing the methods and devices described above . In a preferred embodiment, paclitaxel is administered as an aqueous solution or in a sustained-release form (*i.e.*, in association with a polymeric or nonpolymeric carrier). In a particularly preferred embodiment, paclitaxel (or an analogue or derivative thereof) is administered in a sustained-release preparation (*e.g.*, PLA microspheres or hyaluronic acid microspheres containing paclitaxel) at a dose of 1 to 50 mg/m^2 which disperses throughout the pericardial space. The sustained-release preparation is designed to release therapeutic levels of paclitaxel over a period of several days to several months. This dose is then repeated 1 to 4 times per year (or as required) to slow the progression of atherosclerosis or stabilize atherosclerotic plaques.

In the case of acelerated atherosclerosis associated with cardiac transplantation, paclitaxel can be administered in a slow release form that delivers a total dose of 1 to 75 mg/m^2 (preferably 10 to 50 mg/m^2) of drug over a selected period of time. The drug may be administered initially as a paste or gel placed at the time of transplant surgery. Subsequent drug administration is then achieved in the manner described in the previous paragraph. A preferred carrier for the drug is hyaluronic acid as this agent, in combination with paclitaxel, may help reduce the incidence of post-surgical pericardial adhesions.

Regardless of the methods employed, the anti-microtubule agent should be capable of reducing the signs and/or symptoms of coronary artery obstruction such as chest pain (angina), syncope, dyspnea, orthopnea, radiating pain (shoulder, arm pain), nausea, or diaphoresis. The treatment may also preserve coronary artery luminal diameter/area, reduce the incidence of total occlusion (myocardial infarction), or preserve myocardial perfusion as demonstrated by standard cardiac function and radiographic methods.

TREATMENT OF TRANSPLANT REJECTION

In the case of organ rejection associated with cardiac transplantation, paclitaxel can be administered in a slow release form that delivers a total dose of 1 to 75 mg/m^2 (preferably 10 to 50 mg/m^2) of drug over a selected period of time. The drug may be administered initially as a paste or gel placed at the time of transplant surgery. Subsequent drug administration is then achieved via direct pericardial injection.

The anti-microtubule agent is injected periodically (*e.g.*, once every few months) directly into the pericardial sac utilizing the methods and devices described previously. In a preferred embodiment, paclitaxel (or an analogue or derivative thereof) is administered in a sustained-release preparation (*e.g.*, PLA microspheres or hyaluronic acid microspheres containing paclitaxel) at a dose of 1 to 50 mg/m^2 which disperses throughout the pericardial space. The sustained-release preparation is designed to release therapeutic levels of paclitaxel over a period of several days to several months. A preferred carrier for the drug is hyaluronic acid as this agent, in combination with paclitaxel, may also help reduce the incidence of post-surgical pericardial adhesions. This dose is then repeated 1 to 4 times per year (or as required) to help prevent transplant rejection.

TREATMENT OF ARTERITIS AND OTHER RHEUMATIC CONDITIONS AFFECTING THE HEART

Several rheumatic diseases are associated with cardiac manifestations, such as arteritis, systemic lupus erythematosis, and rheumatoid arthritis. As described herein, anti-microtubule agents such as paclitaxel can be utilized in the systemic treatment of rheumatic diseases. Intrapericardial administration may be particularly effective in the management of pericarditis, coronary arteritis (*e.g.*, Kowasaki's disease, polyarteritis) and myocarditis

associated with these conditions. Local administration of an anti-microtubule agent into the pericardial sac can result in increased drug levels at the site of the disease while decreasing systemic exposure to the agent.

Briefly, the anti-microtubule agent is injected periodically (*e.g.*, once every few months) directly into the pericardial sac utilizing the methods and devices described previously. In a preferred embodiment, paclitaxel (or an analogue or derivative thereof) is administered in a sustained-release preparation (*e.g.*, PLA microspheres or hyaluronic acid microspheres containing paclitaxel) at a dose of 1 to 50 mg/m^2 which disperses throughout the pericardial space. The sustained-release preparation is designed to release therapeutic levels of paclitaxel over a period of several days to several months. A preferred carrier for the drug is hyaluronic acid as this agent, in combination with paclitaxel, may also help reduce the formation of pericardial adhesions. This dose is then repeated 1 to 4 times per year (or as required) to help prevent transplant rejection.

It should be noted that certain infections leading to pericarditis and/or myocarditis could also benefit from anti-microtubule therapy. In particular, certain paracytic infections (*e.g.*, malaria) are responsive to anti-microtubule drugs such as paclitaxel.

TREATMENT VALVULAR STENOSIS AND SHUNT RESTENOSIS

Cardiac surgery is often performed to replace the patient's leaking or obstructed valves with porcine or mechanical heart valves. A complication of this procedure is that scarring of the annular ring of the valve can lead to narrowing or stenosis of the valve and loss of function. In a similar manner, shunts are artificial connections created between chambers of the heart that allow blood to pass between chambers to reduce pressure in one of the chambers. These openings can also scar shut (or stenose) and limit the effectiveness of the procedure. As described herein, anti-microtubule agents, such as paclitaxel can be utilized in the prevention of scar tissue formation.

In a preferred embodiment, paclitaxel can be administered in a slow release form that delivers a total dose of 1 to 75 mg/m^2 (preferably 10 to 50 mg/m^2) of drug over a selected period of time. The drug may be administered initially as a paste or gel placed at the

time of valvular or shunt surgery. Subsequent drug administration is then achieved via direct pericardial injection.

The anti-microtubule agent is then injected periodically (*e.g.*, once every few months) directly into the pericardial sac utilizing the methods and devices described previously. In a preferred embodiment, paclitaxel (or an analogue or derivative thereof) is administered in a sustained-release preparation (*e.g.*, PLA microspheres or hyaluronic acid microspheres containing paclitaxel) at a dose of 1 to 50 mg/m^2 which disperses throughout the pericardial space. The sustained-release preparation is designed to release therapeutic levels of paclitaxel over a period of several days to several months. A preferred carrier for the drug is hyaluronic acid as this agent, in combination with paclitaxel, may also help reduce the incidence of post-surgical pericardial adhesions. This dose is then repeated 1 to 4 times per year (or as required) to help prevent stenosis of the valve or shunt.

The anti-microtubule agent should be capable of reducing the signs and/or symptoms of valvular obstruction or shunt obstruction such as chest pain (angina), syncope, dyspnea, orthopnea, paroxysmal nocturnal dyspnea, radiating pain (shoulder, arm pain), nausea, congestive heart failure, pulmonary edema, or hepatosplenomegaly.

TREATMENT OF CARDIAC ADHESIONS FOLLOWING SURGERY

Cardiac surgery for valve replacement or coronary artery bypass grafting (CABG) is an extremely common surgical procedure. A complication of this procedure is that scarring of the pericardium can lead to the formation of adhesions (+/- effusions) that impact negatively on cardiac contractility and function. As described in the following examples, anti-microtubule agents such as paclitaxel can be utilized in the prevention of surgical adhesion formation.

In a preferred embodiment, paclitaxel can be administered intraoperatively in a slow release paste or gel that delivers a total dose of 1 to 75 mg/m^2 (preferably 10 to 50 mg/m^2) of drug over a selected period of time. Subsequent drug administration is then achieved via direct pericardial injection.

The anti-microtubule agent is then injected periodically (*e.g.*, once every few months) directly into the pericardial sac utilizing the methods and devices described

previously. In a preferred embodiment, paclitaxel (or an analogue or derivative thereof) is administered in a sustained-release preparation (*e.g.*, PLA microspheres or hyaluronic acid microspheres containing paclitaxel) at a dose of 1 to 50 mg/m^2 which disperses throughout the pericardial space. The sustained-release preparation is designed to release therapeutic levels of paclitaxel over a period of several days to several months. A preferred carrier for the drug is hyaluronic acid as this agent, in combination with paclitaxel, may also help reduce the incidence of post-surgical pericardial adhesions. This dose is then repeated 1 to 4 times per year (or as required) to help prevent stenosis of the valve or shunt.

The anti-microtubule agent should be capable of reducing the signs and/or symptoms of pericardial adhesions such as chest pain, syncope, dyspnea, congestive heart failure, pericardial friction rubs, and decreased cardiac output.

TREATMENT OF MALIGNANT PERICARDIAL EFFUSIONS

Primary or metastatic malignancy can affect the heart leading to malignant pericarditis and pericardial effusions. Given that anti-microtubule agents are potent chemotherapeutic drugs, pericardial administration of these agents can be useful for palliative relief of this condition.

The anti-microtubule agent is injected periodically (*e.g.*, once every few days to months) directly into the pericardial sac utilizing the methods and devices described previously. In a preferred embodiment, paclitaxel (or an analogue or derivative thereof) is administered in a sustained-release preparation (*e.g.*, PLA microspheres or hyaluronic acid microspheres containing paclitaxel) at a dose of 50 to 350 mg/m^2 which disperses throughout the pericardial space. The sustained-release preparation is designed to release therapeutic chemotherapeutic levels of paclitaxel over a period of several days to several months. This dose is then repeated as required to help provide palliative relief to the patient.

TREATMENT OF CARDIAC RHYTHM DISORDERS

Numerous cardiac rhythym disorders have been described that lead to conditions ranging from mild discomfort to sudden cardiac death. Certain rhythm

abnormalities (*e.g.*, ablation of abnormal conduction pathways) may benefit from the local application of cytotoxic anti-microtubule therapy.

As should be readily evident, with any of the embodiments discussed herein, the anti-microtubule agent (*e.g.*, paclitaxel) may be administered along with other therapeutics.

The following examples are offered by way of illustration, and not by way of limitation.

EXAMPLES

EXAMPLES

EXAMPLE 1

MANUFACTURE OF AND USE OF DIBLOCK CO-POLYMERS FOR FORMULATING THE DELIVERY OF ANTI-MICROTUBULE AGENTS.

One formulation for paclitaxel is comprised of amphiphilic diblock copolymers which in aqueous solutions form micelles consisting of a hydrophobic core and a hydrophilic shell in water. Briefly, diblock copolymers of poly(DL-lactide)-block-methoxy polyethylene glycol (PDLLA-MePEG), polycaprolactone-block methoxy polyethylene glycol (PCL-MePEG) and poly(DL-lactide-co-caprolactone)-block-methoxy polyethylene glycol (PDLLACL-MePEG) can be synthesized using a bulk melt polymerization procedure, or similar methods. Briefly, given amounts of monomers DL-lactide, caprolactone and methoxy polyethylene glycols with different molecular weights were heated (130°C) to melt under the bubbling of nitrogen and stirred. The catalyst stannous octoate (0.2% w/w) was added to the molten monomers. The polymerization was carried out for 4 hours. The molecular weights, critical micelle concentrations and the maximum paclitaxel loadings were measured with GPC, fluorescence, and solubilization testing, respectively. High paclitaxel carrying capacities were obtained. The ability of solubilizing paclitaxel depends on the compositions and concentrations of the copolymers. PDLLA-MePEG gave the most stable solubilized paclitaxel.

The strong association within the internal core of the polymeric micelles presents a high capacity environment for carrying hydrophobic drugs such as paclitaxel. The drugs can be covalently coupled to block copolymers to form a micellar structure or can be physically incorporated within the hydrophobic cores of the micelles. The mechanisms of drug release from the micelles include diffusion from the core and the exchange between the single polymer chains and the micelles. The small size of the micelles (normally less than 100 nm) will eliminate the difficulties associated with injecting larger particles.

EXAMPLE 2

MODIFICATION OF PACLITAXEL RELEASE FROM THERMOPASTE USING PDLLA-PEG-PDLLA AND LOW MOLECULAR WEIGHT POLY(D,L, LACTIC ACID)

A. Preparation of PDLLA-PEG-PDLLA and low molecular weight PDLLA

DL-lactide was purchased from Aldrich. Polyethylene glycol (PEG) with molecular weight 8,000, stannous octoate, and DL-lactic acid were obtained from Sigma. Poly-ε-caprolactone (PCL) with molecular weight 20,000 was obtained from Birmingham Polymers (Birmingham, AL). Paclitaxel was purchased from Hauser Chemicals (Boulder, CO). Polystyrene standards with narrow molecular weight distributions were purchased from Polysciences (Warrington, PA). Acetonitrile and methylene chloride were HPLC grade (Fisher Scientific).

The triblock copolymer of PDLLA-PEG-PDLLA was synthesized by a ring opening polymerization. Monomers of DL-lactide and PEG in different ratios were mixed and 0.5 wt% stannous octoate was added. The polymerization was carried out at 150°C for 3.5 hours. Low molecular weight PDLLA was synthesized through polycondensation of DL-lactic acid. The reaction was performed in a glass flask under the conditions of gentle nitrogen purge, mechanical stirring, and heating at 180°C for 1.5 hours. The PDLLA molecular weight was about 800 measured by titrating the carboxylic acid end groups.

B. Manufacture of paste formulations

Paclitaxel at loadings of 20% or 30% was thoroughly mixed into either the PDLLA-PEG-PDLLA copolymers or blends of PDLLA:PCL 90:10, 80:20 and 70:30 melted

at about 60°C. The paclitaxel-loaded pastes were weighed into 1 ml syringes and stored at 4°C.

C. *In vitro* release of paclitaxel

A small pellet of 20% paclitaxel-loaded PDLLA:PCL paste (about 2 mg) or a cylinder (made by extruding molten paste through a syringe) of 20% paclitaxel-loaded PDLLA-PEG-PDLLA paste were placed into capped 14 ml glass tubes containing 10 ml phosphate buffered saline (pH 7.4) with 0.4 g/L albumin. The tube was incubated at 37°C with gentle rotational mixing. The supernatant was withdrawn periodically for paclitaxel analysis and replaced with fresh PBS/albumin buffer. The supernatant (10 ml) was extracted with 1 ml methylene chloride. The water phase was decanted and the methylene chloride phase was dried under a stream of nitrogen at 60°C. The dried residue was reconstituted in a 40:60 water:acetonitrile mixture and centrifuged at 10,000g for about 1 min. The amount of paclitaxel in the supernatant was then analyzed by HPLC. HPLC analysis was performed using a 110A pump and C-8 ultrasphere column (Beckman), and a SPD-6A UV detector set at 232 nm, a SIL-9A autoinjector and a C-R3A integrator (Shimadzu). The injection volume was 20 µl and the flow rate was 1 ml/minute. The mobile phase was 58% acetonitrile, 5% methanol, and 37% distilled water.

D. Results and Discussion

The molecular weight and molecular weight distribution of PDLLA-PEG-PDLLA, relative to polystyrene standards, were measured by GPC. The intrinsic viscosity of the copolymer in $CHCl_3$ at 25°C was determined using a Canon-Fenske viscometer. The molecular weight and intrinsic viscosity decreased with increasing PEG content. The polydispersities of PDLLA-PEG-PDLLA with PEG contents of 10% - 40% were from 2.4 to 3.5. However, the copolymer with 70% PEG had a narrow molecular weight distribution with a polydispersity of 1.21. This might be due to a high PEG content reducing the chance of side reactions such as transesterfication which results in a wide distribution of polymer molecular weights. Alternatively, a coiled structure of the hydrophobic-hydrophilic block copolymers may result in an artificial low polydispersity value.

The PEG and PDLLA-PEG-PDLLA with PEG contents of 70% and 40% showed endothermic peaks with decreasing enthalpy and temperature as the PEG content of the copolymer decreased. The endothermic peaks in the copolymers of 40% and 70% PEG were probably due to the melting of the PEG region, indicating the occurrence of phase separation. While pure PEG had a sharp melting peak, the copolymers of both 70% and 40% PEG showed broad peaks with a distinct shoulder in the case of 70% PEG. The broad melting peaks may have resulted from the interference of PDLLA with the crystallization of PEG. The shoulder in the case of 70% PEG might represent the glass transition of the PDLLA region. No thermal changes occurred in the copolymers with PEG contents of 10%, 20% and 30% in a temperature range of 10 - 250°C, indicating that no significant crystallization (therefore may be the phase separation) had occurred.

DSC thermograms of PDLLA:PCL (70:30, 80:20, 90:10) blends without paclitaxel or with 20% paclitaxel showed an endothermic peak at about 60°C, resulting from the melting of PCL. Due to the amorphous nature of the PDLLA and its low molecular weight (800), melting and glass transitions of PDLLA were not observed. No thermal changes due to the recrystallization or melting of paclitaxel was observed.

PDLLA-PEG-PDLLA copolymers of 20% and 30% PEG content were selected as optimum formulation materials for the paste for the following reasons: PDLLA-PEG-PDLLA of 10% PEG could not be melted at a temperature of about 60°C; the copolymers of 40% and 70% PEG were readily melted at 60°C, and the 20% and 30% PEG copolymer became a viscous liquid between 50°C to 60°C; and the swelling of 40% and 70% PEG copolymers in water was very high resulting in rapid dispersion of the pastes in water.

EXAMPLE 3

PROCEDURE FOR PRODUCING NANOPASTE

Nanopaste is a suspension of microspheres in a hydrophilic gel. Within one aspect of the invention, the gel or paste can be smeared over tissue as a method of locating drug-loaded microspheres close to the target tissue. Being water based, the paste soon becomes diluted with bodily fluids causing a decrease in the stickiness of the paste and a

tendency of the microspheres to be deposited on nearby tissue. A pool of microsphere encapsulated drug is thereby located close to the target tissue.

Reagents and equipment which were utilized within these experiments include glass beakers, Carbopol 925 (pharmaceutical grade, Goodyear Chemical Co.), distilled water, sodium hydroxide (1 M) in water solution, sodium hydroxide solution (5 M) in water solution, microspheres in the 0.1 lm to 3 lm size range suspended in water at 20% w/v (see previous).

1. PREPARATION OF 5% W/V CARBOPOL GEL

A sufficient amount of carbopol was added to 1 M sodium hydroxide to achieve a 5% w/v solution. To dissolve the carbopol in the 1 M sodium hydroxide, the mixture was allowed to sit for approximately one hour. During this time period, the mixture was stirred and, after one hour, the pH was adjusted to 7.4 using 5 M sodium hydroxide until the carbopol was fully dissolved. Once a pH of 7.4 was achieved, the gel was covered and allowed to sit for 2 to 3 hours.

2. PROCEDURE FOR PRODUCING NANOPASTE

A sufficient amount of 0.1 μm to 3 μm microspheres was added to water to produce a 20% suspension of the microspheres. Carbopol gel (8 ml of the 5% w/v) was placed into a glass beaker and 2 ml of the 20% microsphere suspension was added. The mixture was stirred to thoroughly disperse the microspheres throughout the gel. This mixture was stored at 4°C.

EXAMPLE 4

POLYMERIC COMPOSITIONS WITH INCREASED CONCENTRATIONS OF PACLITAXEL

PDLLA-MePEG and PDLLA-PEG-PDLLA are block copolymers with hydrophobic (PDLLA) and hydrophilic (PEG or MePEG) regions. At appropriate molecular weights and chemical composition, they may form tiny aggregates of hydrophobic PDLLA

core and hydrophilic MePEG shell. Paclitaxel can be loaded into the hydrophobic core, thereby providing paclitaxel with an increased "solubility."

A. Materials

D,L-lactide was purchased from Aldrich, Stannous octoate, poly (ethylene glycol) (mol. wt. 8,000), MePEG (mol. wt. 2,000 and 5,000) were from Sigma. MePEG (mol. wt. 750) was from Union Carbide. The copolymers were synthesized by a ring opening polymerization procedure using stannous octoate as a catalyst (Deng et al., *J. Polym. Sci., Polym. Lett. 28*:411-416, 1990; Cohn et al., *J. Biomed. Mater. Res. 22*: 993-1009, 1988).

For synthesizing PDLLA-MePEG, a mixture of DL-lactide/MePEG/stannous octoate was added to a 10 milliliter glass ampoule. The ampoule was connected to a vacuum and sealed with flame. Polymerization was accomplished by incubating the ampoule in a 150 °C oil bath for 3 hours. For synthesizing PDLLA-PEG-PDLLA, a mixture of D,L-lactide/PEG/stannous octoate was transferred into a glass flask, sealed with a rubber stopper, and heated for 3 hours in a 150°C oven. The starting compositions of the copolymers are given in Tables 1 and 2. In all the cases, the amount of stannous octoate was 0.5% - 0.7%.

B. Methods

The polymers were dissolved in acetonitrile and centrifuged at 10,000 g for 5 minutes to discard any non-dissolvable impurities. Paclitaxel acetonitrile solution was then added to each polymer solution to give a solution with paclitaxel (paclitaxel + polymer) of 10% wt. The solvent acetonitrile was then removed to obtain a clear paclitaxel/PDLLA-MePEG matrix, under a stream of nitrogen and 60°C warming. Distilled water, 0.9% NaCl saline, or 5% dextrose was added at four times weight of the matrix. The matrix was finally "dissolved" with the help of vortex mixing and periodic warming at 60°C. Clear solutions were obtained in all the cases. The particle sizes were all below 50 nm as determined by a submicron particle sizer (NICOMP Model 270). The formulations are given in Table 1.

Table 1. Formulations of Paclitaxel/PDLLA-MePEG*

PDLLA-MePEG	Dissolving Media	Paclitaxel Loading (final paclitaxel concentrate)
2000/50/50	water	10% (20 mg/ml)
2000/40/60	water	10% (20 mg/ml)
2000/50/50	0.9% saline	5% (10 mg/ml)
2000/50/50	0.9% saline	10% (20 mg/ml)
2000/50/50	5% dextrose	10% (10 mg/ml)
2000/50/50	5% dextrose	10% (20 mg/ml)

In the case of PDLLA-PEG-PDLLA (Table 2), since the copolymers cannot dissolve in water, paclitaxel and the polymer were co-dissolved in acetone. Water or a mixture of water/acetone was gradually added to this paclitaxel polymer solution to induce the formation of paclitaxel/polymer spheres.

Table 2. Composition of PDLLA-PEG-PDLLA

Copolymer Name	Wt. of PEG (g)	Wt. of DL-lactide (g)
PDLLA-PEG-PDLLA 90/10	1	9
PDLLA-PEG-PDLLA 80/20	2	8
PDLLA-PEG-PDLLA 70/30	3	7
PDLLA-PEG-PDLLA 60/40	4	6
PDLLA-PEG-PDLLA 30-/70	14	6

* PEG molecular weight. 8,000.

C. Results

Many of the PDLLA-MePEG compositions form clear solutions in water, 0.9% saline, or 5% dextrose, indicating the formation of tiny aggregates in the range of nanometers. Paclitaxel was loaded into PDLLA-MePEG micelles successfully. For example, at % loading (this represents 10 mg paclitaxel in 1 ml paclitaxel/PDLLA-MePEG/aqueous

system), a clear solution was obtained from 2000-50/50 and 2000-40/60. The particle size was about 60 nm.

EXAMPLE 5

MANUFACTURE OF MICELLAR PACLITAXEL

Poly(DL-lactide)-block-methoxypolyethylene glycol (PDLLA-block-MePEG) with a MePEG molecular weight of 2000 and a PDLLA:MePEG weight ratio 40:60 is used as the micellar carrier for the solubilization of paclitaxel. PDLLA-MePEG 2000-40/60 (polymer) is an amphiphilic diblock copolymer that dissolves in aqueous solutions to form micelles with a hydrophobic PDLLA core and hydrophilic MePEG shell. Paclitaxel is physically trapped in the hydrophobic PDLLA core to achieve the solubilization.

The polymer was synthesized from the monomers methoxypolyethylene glycol and DL-lactide in the presence of 0.5% w/w stannous octoate through a ring opening polymerization. Stannous octoate acted as a catalyst and participated in the initiation of the polymerization reaction. Stannous octoate forms a number of catalytically reactive species which complex with the hydroxyl group of MePEG and provide an initiation site for the polymerization. The complex attacks the DL-lactide rings and the rings open up and are added to the chain, one-by-one, forming the polymer. The calculated molecular weight of the polymer is 3,333.

All reaction glassware was washed and rinsed with Sterile Water for Irrigation, USP, dried at 37°C, followed by depyrogenation at 250°C for at least 1 hour. MePEG (240 g) and DL-lactide (160 g) were weighed and transferred to a round bottom glass flask using a stainless steel funnel. A 2 inch Teflon coated magnetic stir bar was added to the flask. The flask was sealed with a glass stopper and then immersed to the neck in a 140°C oil bath. After the MePEG and DL-lactide melted, 2 ml of 95% stannous octoate (catalyst) was added to the flask. The flask was vigorously shaken immediately after the addition to ensure rapid mixing and then returned to the oil bath. The reaction was allowed to proceed for an additional 6 hours with heat and stirring. The liquid polymer was then poured into a stainless steel tray, covered and left in a chemical fume hood overnight (about 16 hours). The polymer solidified in the tray. The top of the tray was sealed using Parafilm®. The sealed tray

containing the polymer was placed in a freezer at -20 ± 5°C for at least 0.5 hour. The polymer was then removed from the freezer, broken up into pieces and transferred to glass storage bottles and stored refrigerated at 2 to 8°C.

Preparation of a 50 mg/m^2 Dose

Preparation of the bulk and filling of paclitaxel/polymer matrix was accomplished essentially as follows. Reaction glassware was washed and rinsed with Sterile Water for Irrigation USP, and dried at 37°C, followed by depyrogenation at 250°C for at least 1 hour. First, a phosphate buffer (0.08 M, pH 7.6) was prepared. The buffer was dispensed at the volume of 10 ml per vial. The vials were heated for 2 hours at 90°C to dry the buffer. The temperature was then raised to 160°C and the vials dried for an additional 3 hours.

The polymer was dissolved in acetonitrile at 15% w/v concentration with stirring and heat. The polymer solution was then centrifuged at 3000 rpm for 30 minutes. The supernatant was poured off and set aside. Additional acetonitrile was added to the precipitate and centrifuged a second time at 3000 rpm for 30 minutes. The second supernatant was pooled with the first supernatant. Paclitaxel was weighed and then added to the supernatant pool. The solution was brought to the final desired volume with acetonitrile.

The paclitaxel/polymer matrix solution is dispensed into the vials containing previously dried phosphate buffer at a volume of 10 ml per vial. The vials are then vacuum dried to remove the acetonitrile. The paclitaxel/polymer matrix is then terminally sterilized by irradiation with at least 2.5 Mrad Cobalt-60 (Co-60) x-rays.

EXAMPLE 6

MANUFACTURE OF MICROSPHERES

The equipment used for the manufacture of microspheres include: 200 ml water jacketed beaker (Kimax or Pyrex), Haake circulating water bath, overhead stirrer and controller with 2 inch diameter (4 blade, propeller type stainless steel stirrer - Fisher brand), 500 ml glass beaker, hot plate/stirrer (Corning brand), 4 X 50 ml polypropylene centrifuge tubes (Nalgene), glass scintillation vials with plastic insert caps, table top centrifuge (GPR Beckman), high speed centrifuge- floor model (JS 21 Beckman), Mettler analytical balance

(AJ 100, 0.1 mg), Mettler digital top loading balance (AE 163, 0.01 mg), automatic pipetter (Gilson). Reagents include PCL (mol. wt. 10,000 to 20,000; Polysciences, Warrington Pennsylvania, USA), "washed" (see later method of "washing") EVA, PLA (mol. wt. 15,000 to 25,000; Polysciences), polyvinyl alcohol ("PVA" - mol. wt. 124,000 to 186,000; 99% hydrolyzed; Aldrich Chemical Co., Milwaukee, Wisconsin, USA), DCM or "methylene chloride"; HPLC grade Fisher scientific, and distilled water.

A. Preparation of 5% (w/v) Polymer Solutions

PCL (1.00 g) or PLA, or 0.50 g each of PLA and washed EVA was weighed directly into a 20 ml glass scintillation vial. Twenty milliliters of DCM was then added. The vial was capped and stored at room temperature (25°C) for one hour (occasional shaking may be used), or until all the polymer was dissolved. The solution may be stored at room temperature for at least two weeks.

B. Preparation of 5% (w/v) Stock Solution of PVA

Twenty-five grams of PVA was weighed directly into a 600 ml glass beaker and 500 ml of distilled water was added, along with a 3 inch Teflon coated stir bar. The beaker was covered with glass to decrease evaporation losses, and placed into a 2000 ml glass beaker containing 300 ml of water. The PVA was stirred at 300 rpm at 85°C (Corning hot plate/stirrer) for 2 hours or until fully dissolved. Dissolution of the PVA was determined by a visual check; the solution should be clear. The solution was then transferred to a glass screw top storage container and stored at 4°C for a maximum of two months. The solution, however must be warmed to room temperature before use or dilution.

C. Procedure for Producing Microspheres

Based on the size of microspheres being made (see Table 1), 100 ml of the PVA solution (concentrations given in Table 1) was placed into the 200 ml water jacketed beaker. Haake circulating water bath was connected to this beaker and the contents were allowed to equilibrate at 27°C (+/-1°C) for 10 minutes. Based on the size of microspheres being made (see Table I), the start speed of the overhead stirrer was set, and the blade of the overhead stirrer placed half way down in the PVA solution. The stirrer was then started, and

10 ml of polymer solution (polymer solution used based on type of microspheres being produced) was then dripped into the stirring PVA over a period of 2 minutes using a 5 ml automatic pipetter. After 3 minutes the stir speed was adjusted (see Table 1), and the solution stirred for an additional 2.5 hours. The stirring blade was then removed from the microsphere preparation, and rinsed with 10 ml of distilled water so that the rinse solution drained into the microsphere preparation. The microsphere preparation was then poured into a 500 ml beaker, and the jacketed water bath washed with 70 ml of distilled water, which was also allowed to drain into the microsphere preparation. The 180 ml microsphere preparation was then stirred with a glass rod, and equal amounts were poured into four polypropylene 50 ml centrifuge tubes. The tubes were then capped, and centrifuged for 10 minutes (force given in Table 1). Forty-five milliliters of the PVA solution was drawn off of each microsphere pellet.

TABLE 1

PVA concentrations, stir speeds, and centrifugal force requirements for each diameter range of microspheres.

PRODUCTION STAGE	MICROSPHERE DIAMETER RANGES		
	30 μm to 100 μm	10 μm to 30 μm	0.1 μm to 3 μm
PVA concentration	2.5% (w/v) (*i.e.*,) dilute 5% stock with distilled water	5% (w/v) (*i.e.*, undiluted stock)	3.5% (w/v) (*i.e.*, dilute 5% stock with distilled water
Starting Stir Speed	500 rpm +/- 50 rpm	500 rpm +/- 50 rpm	3000 rpm +/- 200 rpm
Adjusted Stir Speed	500 rpm +/- 50 rpm	500 rpm +/- 50 rpm	2500 rpm +/- 200 rpm
Centrifuge Force	1000 g +/- 100 g (Table top model)	1000 g +/- 100 g (Table top model)	10 000 g +/- 1000 g (High speed model)

Five milliliters of distilled water was then added to each centrifuge tube and vortexed to resuspend the microspheres. The four microsphere suspensions were then pooled into one centrifuge tube along with 20 ml of distilled water, and centrifuged for another 10 minutes (force given in Table 1). This process was repeated two additional times for a total of three washes. The microspheres were then centrifuged a final time, and resuspended in 10

ml of distilled water. After the final wash, the microsphere preparation was transferred into a preweighed glass scintillation vial. The vial was capped, and left overnight at room temperature (25°C) in order to allow the microspheres to sediment out under gravity. Since microspheres which fall in the size range of 0.1 um to 3 um do not sediment out under gravity, they were left in the 10 ml suspension.

D. Drying of 10 μm to 30 μm or 30 μm to 100 μm Diameter Microspheres

After the microspheres sat at room temperature overnight, the supernatant was drawn off of the sedimented microspheres. The microspheres were allowed to dry in the uncapped vial in a drawer for a period of one week or until they were fully dry (vial at constant weight). Faster drying may be accomplished by leaving the uncapped vial under a slow stream of nitrogen gas (flow approx. 10 ml/minute.) in the fume hood. When fully dry (vial at constant weight), the vial was weighed and capped. The labeled, capped vial was stored at room temperature in a drawer. Microspheres were normally stored no longer than 3 months.

E. Determining the Concentration of 0.1 μm to 3 μm Diameter Microsphere Suspension

This size range of microspheres did not sediment out, so they were left in suspension at 4°C for a maximum of four weeks. To determine the concentration of microspheres in the 10 ml suspension, a 200 μl sample of the suspension was pipetted into a 1.5 ml preweighed microfuge tube. The tube was then centrifuged at 10,000 g (Eppendorf table top microfuge), the supernatant removed, and the tube allowed to dry at 50°C overnight. The tube was then reweighed in order to determine the weight of dried microspheres within the tube.

F. Manufacture of Paclitaxel Loaded Microsphere

In order to prepare paclitaxel containing microspheres, an appropriate amount of weighed paclitaxel (based upon the percentage of paclitaxel to be encapsulated) was placed directly into a 20 ml glass scintillation vial. Ten milliliters of an appropriate polymer solution was then added to the vial containing the paclitaxel, which was then vortexed until the paclitaxel dissolved.

Microspheres containing paclitaxel may then be produced essentially as described above in steps (C) through (E).

EXAMPLE 7

Manufacture of Paclitaxel-Loaded Star-Shaped Poly(lactic Acid) (PLA) and Poly(Lactide-co-Glycolic Acid) (PLGA) (PEG) Microspheres

Microspheres containing 5, 10 or 20% paclitaxel in low molecular weight star-shaped PLA and PLGA (M.W. ≈ 10,000 by Gel Permeation Chromatography) were prepared by an oil-in-water emulsification technique. Briefly, the appropriate weights of the paclitaxel and 0.5 polymer were dissolved in 10 ml of dichloromethane and emulsified with a overhead propeller stirrer at the level of 3 (Fisher Scientific) into 100 ml 1% polyvinyl alcohol solution for about 3 hours. The formed microspheres were sieved and dried under vacuum at a temperature below 10°C. Yield of microspheres in the desired size range (53 - 90 μm) was about 50% and the encapsulation efficiency of paclitaxel in microspheres was about 98%.

Release studies were done by placing 2.5 mg of said microspheres in a 15 ml Teflon capped tube (with 10 ml phosphate buffer saline with albumin). Sampling daily (three sampling at the first day) to maintain the sink condition. Release study data showed that paclitaxel was released from the star-shaped microspheres 3 to 10 times faster than the conventional linear PLA and PLGA microspheres.

EXAMPLE 8

Intrapericardial Micellar Paclitaxel Administration in a Porcine Model

Juvenile farm pigs of approximately 20kg weight receive angiography to permit arterial measurement. Balloon injury is then performed alternatively in the LAD or LC at an overstretch ratio of 1.3 to 1 (see Figure 1). The alternative vessel receives a stent injury at the same overstretch ratio. Pericardial access and installation is obtained utilizing PerDUCER devices by Comedicus, Inc. (Minneapolis, MN).

The swine each receive a single infusion of unloaded micelles (control), or, 10 mg or 50 mg paclitaxel loaded micelles. The formulation containing 10 mg of paclitaxel demonstrated a marked improvement as shown in Figures 2 through 6.

EXAMPLE 9

TESTING OF POLYMER BIOCOMPATIBILITY WHEN DELIVERED INTO THE PERICARDIAL CHAMBER OF RABBITS

The objective of this study was to examine the biocompatibility of a number of controlled drug release polymers for the treatment of blood vessel disease when released into the intrapericardial cavity (the cavity between the membrane surrounding the heart and the heart.

Briefly, rabbits were anesthesized and maintained on a respirator with halothane. Following standard surgical procedures to expose the chest cavity, the pericardial sac was identified and punctured with the needle and approximately 1 mL of the polymer in saline was injected. The layers of muscle and skin were then sutured and animals recovered. At the two-week timepoint, animals were euthanized, and the chest cavity opened. Tissues (pericardial membrane, and heart) were examined for adhesion formation and inflammation including erythema, fluid, necrosis, and thickening of the pericardial membrane. Tissues including the heart and pericardial membrane were prepared for histological analysis.

Three groups of rabbits were tested. These included saline (N=4), a hyaluronic acid formulation (N=4) and a Poly-lactic acid microsphere formulation (N=2). The four rabbits injected (1ml) with saline and the 4 animals injected (1ml) with hyaluronic acid paste (20 mg/ml and 40 mg/ml) did not show any sign of toxicity at necropsy. A small area (1x1cm) of white soft material was present on the left ventricle close to the site of injection in the animals injected with microspheres (4mg/ml). The pericardium did not adhere to this tissue. The amount of fat surrounding the heart was remarkable in all animals and prevented thorough inspection of the pericardium at necropsy. Histology of the pericardial tissue following application of these formulations was conducted and did not show evidence of a chronic inflammatory reaction from the polymers.

These results demonstrate that the hyaluronic acid formulation and PLA microspheres are suitable polymers for intrapericardial delivery of drugs.

EXAMPLE 10

SURGICAL ADHESIONS

PROTOCOL

The rabbit uterine horn model was conducted essentially as described by Wiseman *et al.*, 1992 (Journal of Reproductive Medicine, 37: 766-770), with hemostasis. New Zealand female white rabbits were anesthetized and a midline incision made through the skin and the abdominal wall. Both uterine horns were located and exteriorized. Using a French Catheter Scale, the diameter of each uterine horn was measured and recorded. Only those rabbits with uterine horns measuring size 8 to 16, inclusive, on the French scale were used. Using a number 10 scalpel blade, 5 cm lengths of each uterine horn, approximately 1 cm from the uterine bifurcation, were scraped, 40 times per side, until punctuate bleeding. Hemostasis was achieved by tamponade.

Animals were randomized to receive: no treatment (Surgical Control); polymer Vehicle Control; paclitaxel (0.1% in vehicle); and paclitaxel (1% in vehicle). Test agent (0.4 to 2.5 ml) was applied over the horns via an 18 gauge needle. Uterine horns were replaced into the pelvis and the abdominal incision closed.

At 18, 31, 32, 33 and 60 days after surgery, animals were euthanized by intravenous injection of sodium pentobarbital (120 mg/ml; 1 ml/kg). Body weights of the animals were recorded. The abdomen was opened and the surgical site inspected. Adhesions were graded by a blinded observer as follows:

Extent of Adhesions The total length (cm) of each uterine horn involved with adhesions was estimated and recorded.

Tenacity (Severity) of Adhesions Adhesions were grades as 0 (absent), 1.0 (filmy adhesions) and 2.0 (tenacious, requiring sharp dissection).

Degree of Uterine Convolution The degree of uterine convolution was recorded according to the following scale:

No convolution:	Straight lengths of adherent or non-adherent horns which are clearly discerned.
Party convoluted:	Horns have adhesions and 50%-75% of the horn length is entangled preventing discernment of straight portions.
Completely convoluted:	It is impossible to discern uterine anatomy because the horn is completely entangled.

RESULTS

All animals maintained or gained weight during the study period. By inspection, there appeared to be no differences in average weight gain between the groups.

By inspection the extent of adhesion formation did not appear to vary with the time, in each group. Thus data for each group have been pooled. Adhesions formed in surgical controls to an extent consistent with historical data for this model. Paclitaxel exhibited a dose-dependent reduction in the extent of adhesions from 4.781 ± 0.219 cm in the Vehicle Control Group (N=8) to 2.925 ± 0.338 cm ($p<0.05$) and 2.028 ± 0.374 cm ($p<0.01$) in the 0.1% (N=10) and 1% (N=9) paclitaxel groups, respectively (Table 1).

Table 1

Effect of Paclitaxel on Adhesion Formulation in a Rabbit Uterine Horn Model

Group	*Extent*[1]	*Adhesion-Free*[2]	*Convolution*[3]	*N*
B. Vehicle Control	4.781 (0.219)	0/16	3/6/7	8
D. 0.1% paclitaxel	2.925 (0.338)*	0/20	16/2/2†	10
A. 1% paclitaxel	2.028 (0.374)**	0/18	18/0/0‡	9
C. Surgical Control	2.700 (0.407)**	0/20	16/2/2†	10

[1] Length of uterine horn with adhesions, cm (± Standard Error of the Mean)

[2] Number of uterine horns free of adhesions/total

[3] Number of uterine horns with no convolution/partial convolution/full convolution

* $p < 0.05$ (Dunnett's test); $p < 0.01$ *Student's t test) vs Vehicle Control unequal variance

** $p < 0.01$ (Dunnett's test), vs Vehicle Control

† $p = 0.0031$ (Fisher's Exact Test), vs Vehicle Control, Convolution classed as Present/Absent
$x^2 = 8.251$

‡ $p = <0.0001$ (Fisher's Exact Test), vs Vehicle Control, Convolution classed as Present/Absent
$x^2 = 17.07$

The degree of uterine convolution was also reduced in the 0.1% paclitaxel ($p = 0.0031$) and 1% paclitaxel ($p<0.0001$) groups.

EXAMPLE 11

EFFECTS OF MICELLAR PACLITAXEL HYALURONIC GEL IN AN ANIMAL MODEL OF SURGICAL ADHESIONS

The use of micellar paclitaxel hyaluronic acid gel to reduce adhesion formation is examined in the rat cecal abrasion model of surgical adhesions. The formulation would be applicable for application through the intrapericardial method of delivery.

METHODS

The rat cecal abrasion model is a well-established model of surgical adhesions. Male Sprague Dawley rats weighing 300 – 400 g were anaesthetized and maintained on 1.5 – 2% Halothane. The abdomen was shaved and scrubbed with an alcohol-based antiseptic wash, draped and opened with a central laparotomy incision of 3 – 4 cm within a sterile field. The cecum and large bowel were externalized with sterile swabs, and the cecum supported by a sponge such that contents could be evacuated into the large bowel. Both caecal surfaces were then stroked 45 times with a #10 scalpel blade to produce erythema and punctated bleeding. In no case did this treatment yield sustained bleeding requiring ligation. Each stroke spanned most of the caecal diameter (approximately 1 cm), and extended along 1.5 cm of the caecal extremity. After a delivery of a total of 90 strokes, the integrity of the tissue was confirmed, and cecum and large bowel were replaced in the pelvis.

One of two doses of Micellar Paclitaxel, (0.6 mg or 1.2 mg total Paclitaxel, or Hyaluronic acid gel with Micelles alone, were contained in a 3 mL bolus. The formulation was directed by syringe into the abdominal quadrant surrounding the damaged cecum, and the laparotomy was closed in two layers. Animals were warmed and monitored until fully recovered from the anaesthetic, and subsequently housed separately with food and water *ad libitum*, for one week.

EVALUATION OF ADHESIONS

One week following surgery, animals were euthanized with Sodium Pentobarbital and the abdomen opened for examination. Adhesions over, and adjacent to the cecum were rated according to the following findings: 0: No adhesions; 1: Filmy or stranded adhesions connected the cecum with omentum or adjacent intestines; 2: Cohesive adhesions tethered the cecum, which required aggressive blunt dissection; 3: Sharp dissection of adhesions was required to free the cecum from surrounding tissues. Fractions were assigned where a variety of ratings seemed applicable.

RESULTS

The use of hyaluronic acid gel reduced the severity of surgical adhesions by approximately 15%. The addition of micellar paclitaxel to the hyaluronic acid gel resulted in a dose-dependent reduction in the mean severity of adhesions. In addition, the percent of rats

showing an adhesion score greater than 2.5 was reduced by 60% at the higher paclitaxel concentration relative to the non-treated group and by 50% relative to the hyaluronic \acid gel group alone.

Table 1.

Treatment group	Individual ratings	Mean Severity of Adhesions	% of subjects >2.5
No Formulation (n=4)	3.0, 3.0, 3.5, 3.5	3.25	100%
Hyaluronic Acid Gel/Empty Micelles (n=5)	2.75, 2.0, 2.75, 3.0, 3.0	2.7	80%
Hyaluronic Acid Gel/Micellar Paclitaxel 0.6 mg (n=5)	1.25, 2.75, 3.5, 1.75, 2.5	2.35	60%
Hyaluronic Acid Gel/Micellar Paclitaxel 1.2 mg (n=5)	1.25, 1.25, 2.5, 1.75, 2.5	1.85	40%

CONCLUSION

These results suggest that micellar paclitaxel can improve preclinical surgical adhesion outcome in a well-established model of surgical adhesions. Such formulations can also reduce the incidence of surgical adhesions associated with cardiac surgery, when delivered through the intrapericardial route of administration.

EXAMPLE 12

INTRAPERICARDIAL PACLITAXEL DELIVERY INHIBITS NEOINTIMAL PROLIFERATION AND PROMOTES ARTERIAL ENLARGEMENT AFTER PORCINE CORONARY OVERSTRETCH

Catheter-based approaches to intrapericardial (IPC) delivery of therapeutic agents have been recently demonstrated to be feasible. This study examined the effect of IPC instillation of paclitaxel on neointimal proliferation induced by balloon overstretch of porcine coronary arteries.

METHODS

Both paclitaxel and copolymer were dissolved in 0.9% sodium chloride at 50°C±5°C. The solutions were then sterile-filtered and utilized within four hours for

pericardial instillation.

Eighteen juvenile female domestic pigs weighing 23 to 25 kg were used for this study. The animals were divided into three instillation groups: low-dose (LD, 10 mg paclitaxel, n=6); high-dose (HD, 50 mg paclitaxel, n=7); and control group (C, 50 mg copolymer, n=5). Each group had a consistent delivery volume (25 ml). All animals received a normal diet and were housed in similar runs.

EXPERIMENTAL PROTOCOL

All animals were fasted overnight and premedicated with aspirin (325 mg) 24 hours prior to operation. The animals were sedated with an intramuscular combination of ketamine (20 mg/kg), xylazine (2 mg/kg), and atropine (0.05 mg/kg). Anesthesia was initiated with IV sodium pentothal (25 mg/kg). After intubation, the animals were mechanically ventilated using air mixed with oxygen (2 L/min) and isoflurane (2.5%). The ECG and blood pressure were monitored continuously. The operation was carried out under sterile conditions.

All animals underwent coronary overstretch by balloon dilation. Briefly, access to the vascular system was obtained after cutdown of the right carotid or femoral artery. An 8F or 9F introducer sheath was inserted, followed by system heparinization (200 U/kg) and lidocaine (30 mg). An 8F guiding catheter was used to engage the left coronary artery. After intracoronary administered nitroglycerin (200 μg), a left coronary angiogram was performed. The cine frames were immediately converted to digital computer images and the LAD and LCx diameters determined using NIH Image, with the guiding catheter diameter serving as a reference length. A balloon catheter (20 mm long) with a 1.3 balloon: artery diameter ratio was chosen to dilate the target vessel. The balloon inflation was performed three times for 30 seconds, with a 60-second interval intervening. Coronary angiography was finally repeated, the catheters removed, and the cutdown was repaired.

PERCUTANEOUS INTRAPERICARDIAL SPACE DELIVERY

After balloon dilation, a pericardial access device was used for transthoracic insertion of a guidewire into the normal pericardial space. This device (PerDUCER®, Comedicus Inc. Columbia, MN) consists of a needle protectively sheathed with a catheter

bearing a hemispherical-shaped side-hole at its tip. An initial percutaneous tunnel was made below the xiphoid process using a 21-gauge needle introduced nearly parallel to the skin surface, after which a 0.038 inch diameter guidewire and introducer sheath were placed under fluoroscopic guidance into the mediastinum over the anterior pericardium. The sheathed needle device was inserted through an introducer sheath and positioned on the anterior outer surface of the pericardial sac, which was drawn into the hemispherical-shaped tip by manual suction and pierced by the needle. Finally, a 0.018 inch guidewire was placed through the needle lumen and advanced several cm to confirm confinement within the pericardial space. After removal of the needle, a 4F hydrophilic-coated dilator catheter was inserted over the wire. Following wire removal, successful intrapericardial tip placement was tested by contrast injection into the pericardial space; and twenty-five mls of either paclitaxel or copolymer solution was delivered over five minutes into the pericardial sac. The catheter was removed, the cutaneous puncture was sutured, and the animals allowed to recover.

At 28 days after the procedure, the animals were sedated and anesthetized, as previously described. The final coronary angiography was performed after heparin (200 U/kg) administration. The animals were then killed by a lethal dose of pentobarbital (65 mg/kg). Immediately after euthanasia, the heart and pericardial tissue were harvested, and the coronary arteries were perfusion-fixed with 10% buffered zinc formalin for 15 to 20 minutes at 80 mm Hg pressure.

TISSUE PREPARATION AND IMMUNOCYTOCHEMICAL STAINING

The pericardial cavity was inspected before the LAD and LCx were dissected from the heart. Gross pericardial space adhesion was quantified using a modification of the scoring system described by Hurewitz et al. The grades were assigned as: 0=normal, 1= focal thin adhesions, 2=diffuse widespread adhesions, 3=complete obliteration of the pericardial space. After paraffin embedding and sectioning, pericardial tissue was stained with hematoxylin-eosin and Masson's trichrome. Mesothelial cells on the parietal pericardium were graded as either absent or present. The thickness of the visceral pericardium was measured at four sites overlying the left and right atria and ventricles.

To examine the entire LAD and LCx vessel lengths, the vessels were sectioned at 3 mm intervals from the proximal to distal end, and embedded in paraffin using standard

histological techniques. Paraffin sections were cut at 6 μm, affixed to glass microscope slides, and stained with hematoxylin-eosin and Verhoeff-Van Gieson's reagents. Immunohistochemical analysis was performed on selected segments using primary antibodies including anti-smooth muscle α-actin (1:1000, Dako), von Willebrand factor (vWF, 1:600, Dako) and anti-matrix metalloproteinase antibodies (MMP-2, 1:100; Oncogene). Secondary antibody binding was revealed by avidin complex method, with a staining reaction performed using 3,3'-diaminobenzidine (DAB) solution (Sigma). Nuclei were counterstained with hematoxylin or methyl green. Endogenous peroxidase activity was blocked with 3% H_2O_2 solution for 5 minutes. Negative control stains were generated using nonimmune serum instead of primary antibody.

Apoptotic cells were detected by the Klenow fragment end labeling method, using a commercially available kit (Oncogene). Briefly, after deparaffinization, the tissue sections were treated with 20 μg/ml proteinase K/10 mM Tris/HCL, pH 8.0, for 10 minutes. After rinsing in 1×TBS, the Klenow labeling reaction mixture was added. In each experiment, a positive and negative control was included. The positive control was treated with DNaseI (1 mg/ml, 20 minutes, RT) to induce DNA strand breaks; the negative control was stained only with Klenow labeling reaction mix (without Klenow enzyme).

MORPHOMETRIC ANALYSIS

Morphometric measurements were performed using a light microscope (Olympus) at low power (x2.5 microscopic magnification) linked to a color video camera (Sony) and a computer-interfaced image analysis system with NIH Image software. This allowed the manual selection and delineation of artery areas. The endoluminal length (ELL), the circumference bounded by internal elastic lamina (IEL) and the external elastic lamina (EEL) were traced by hand, and the luminal and intimal areas were determined automatically. Fracture length (FL) was defined as the arc length between the two fracture points of the internal elastic lamina. Intimal area (IA) was measured directly. Maximal intimal thickness (MIT) was defined as the maximal distance between the lumen and EEL, while maximal adventitial thickness (MAT) was the analogous length between EEL and adventitia. The percent stenosis (%) was described as the histologic lumen diameter at the site of maximal stenosis divided by the pre-angioplasty luminal diameter determined at the midpoint of the

target segment.

CELL COUNTING

Analysis was done on each cross-section with hematoxylin-stained nuclei of neointima and media cells under ×40 microscopic magnification. Neointima and media cells were counted using a validated method. Briefly, randomly selected areas encompassing 20% to 40% of the total neointimal cross-sectional area were counted. The cells within the media were counted in five regions defined as follows: region 1 and 2, comprised of the two medial ends adjacent to the medial tear; region 3, the site 180° opposite to the neointimal mass; and regions 4 and 5, at 90° radials with respect to the neointima. Cell density (cells/mm^2) was used to determine total numbers in the neointimal and medial areas.

Apoptotic cells in the neointima or media were scored as positive when showing morphologic features characteristic of apoptosis as well as positive Klenow labeling. Cells with cytoplasmic, but not nuclear staining, were scored as negative cells. The apoptosis percentage was determined for each vessel layer.

STATISTICS

Results are presented as mean±SEM. An unpaired *t*-test was used to compare the three group histomorphometric measurement data. Differences are considered significant at $p < 0.05$. All statistical calculations were performed using the SigmaStat™ software package.

RESULTS

Pericardial instillation was well tolerated by all animals. No complications developed during these procedures, and no clinical evidence of paclitaxel-related toxicity was noted.

BASELINE ANGIOGRAPHIC CHARACTERISTICS

The artery diameters before balloon dilation (C, 2.65±0.12 mm; LD, 2.51±0.13 mm; HD, 2.47±0.12 mm; p = NS) and the balloon/artery ratios (C, 1.34±0.02; LD, 1.32±0.03; HD, 1.32±0.02; p = NS) were no different among control, high-dose, and low-dose groups.

PERICARDIAL TISSUE AND CONTENTS

The gross and histological changes of the pericardium after IPC paclitaxel delivery are summarized in Table 1. Intrapericardial adhesions were entirely absent in the C and LD groups, except for three pigs with a few thin adhesions limited to the puncture site (1 pig in control, 2 pigs in LD group). Biochemical parameters were measured in the pericardial fluid of a subset of the LD and C groups, and were within normal ranges. Macroscopic scoring of the pericardial space adhesions, confirmed that the high-dose, but not the low-dose group, was significantly different from control (Adhesion scores C, 0.20±0.20; LD, 0.33±0.21; and HD, 2.57±0.20; $p < .001$ for HD vs. C or LD). Microscopically, the C and LD groups had entirely intact mesothelial layers, with multilayering noted in some parts of the pericardium. In addition to the intracavitary adhesions, the pericardium of the HD group was demonstrably thicker as compared to both the C and LD groups, with an increase found in the dense connective tissue of both parietal and visceral layers. The interlaminar adhesion tissue displayed fibrin and collagen deposition as well as infiltration with mononuclear cells. Cells staining positively for SMC α-actin expression were found dispersed throughout the connective tissue of the visceral pericardium in all groups and displayed greatest intensity of staining in the high-dose group.

MORPHOMETRIC ANALYSIS OF ARTERIES

IPC paclitaxel delivery significantly inhibited neointimal proliferation. Table 2 displays morphometric data for vessels comprising each group. The extent of vessel injury, expressed as an injury index (FL/FL+IEL) was equivalent for each of the three groups (C, 0.21±0.02; LD, 0.22±0.03; and HD, 0.21±0.01; p = NS). The neointimal response was linearly correlated with the degree of vessel injury in the control as well as the experimental groups (Fig. 7, C, $R^2 = 0.69$; HD, $R^2 = 0.66$; LD, $R^2 = 0.44$), but the slope is markedly diminished for vessels receiving paclitaxel at either dose. The absolute neointimal area (Fig. 8A) was significantly smaller in both experimental groups (LD, 0.47±0.04 and HD, 0.51±0.06 mm^2, p = NS) when compared with the control group (0.79±0.07 mm^2, $p < .001$). The neointimal area normalized to FL was also significantly smaller for both treatment groups (LD, 0.32±0.02 and HD, 0.39±0.04) than in the control group (0.68±0.03, $p < .001$). Similarly, the maximal intimal and adventitial thicknesses were lower in both paclitaxel

groups than the C group ($p < .001$). However, the medial area did not differ among the groups. Both paclitaxel groups evidenced outward vascular remodeling in comparison with the control group, with the external elastic lamina (EEL) circumference and enclosed area found to be significantly larger in the LD and HD group (Table 2).

The degree of luminal occlusion, expressed as % stenosis, was significantly reduced in both treated groups (LD, 10±0.02%; and HD, 22±0.03%; versus C 38±0.03%, $p < .001$) (Fig. 8B)), for relative stenosis reductions of 74% in the LD and 42% in the HD groups. The marked reduction in luminal occlusion by paclitaxel is dominantly achieved by its effects on vessel remodeling as expressed in terms of EEL circumference. Comparative evaluation of the area contributions of the decreased neointima and the increased vessel circumference shows that the latter is in fact responsible for 70-80 % of the luminal expansion noted relative to control vessels.

CELL QUANTIFICATION AND IMMUNOHISTOCHEMISTRY

Medial cell density was not measurably different among the C (3983±128 cells/mm^2), LD (3875±244 cells/mm^2), and HD (4089±422 cells/mm^2) groups. However, the neointimal cell density in the HD group (3571±128 cells/mm^2) was significantly lower than C (4574±201 cells/mm^2) and LD (4196±120 cells/mm^2) groups ($p < .001$). The Klenow-positive cells were predominately detected in the neointima, with very few cells found in the media or adventitia. Most staining cells also demonstrated hyperchromatic and fragmented nuclei. Some had histologically normal nuclei, possibly representing cells in an early apoptotic phase. There were no significant differences between the C and LD groups, but the HD group had significantly greater percentage of apoptotic cells than either of these groups (C, 1.72%; LD, 2.31%; versus HD, 7.07%; $p < .0001$).

Immunohistochemical staining demonstrated that neointimal cells were predominantly immunoreactive for α-actin in all groups. The neointima was composed of spindle-shaped cells and a large amount of loose extracellular matrix, where the cells had the appearance of a synthetic phenotype with looser organization than found in media. At 28 days after balloon injury, complete vessel re-endothelialization had been achieved in most vessel segments of all three groups, as measured by positive vWF staining. Since paclitaxel has been known to alter the expression of MMP-2 in other systems, we investigated the

presence of such modulation following IPC paclitaxel delivery. In control, as well as LD vessels, MMP-2 immunoreactivity was found in endothelial cells, neointima, and media. Conversely, there was generally diminished MMP-2 staining in all vessel layers in the HD sections. MMP-2 staining was not characteristically present in the adventitia of any groups.

The present study demonstrates that a single-dose of paclitaxel delivered into the pericardial space results in significant reduction of restenosis after balloon injury of the porcine coronary artery, which is mediated both by reduced neointimal formation and enhanced arterial enlargement. The 10 mg dosage appeared to produce more optimal results, although favorable effects were present at both doses. The clear effect identified *in vivo* 28 days after a single dose is remarkable, and likely may relate to the high affinity of paclitaxel for its specific intracellular target sites on microtubules, as well as its hydrophobic nature, both of which will favor slow redistribution following placement at a particular site. The fluid reservoir formed by the pericardial sac would also be expected to contribute to the persistence of locally effective concentrations of agents delivered by an IPC route.

The vessel circumference evaluated at the external elastic lamina becomes remarkably larger following paclitaxel therapy (C, 7.04±0.22; versus HD, 7.71±0.23; and LD, 8.12±0.18 mm, $p = .017$). Such an effect, found in the context of a conserved medial area, reflects a modulated disposition of tissue mass consistent with altered vascular remodeling in comparison with the control group. The associated finding of reduced adventitial thickness in this study provokes the hypothesis that a decrease in adventitial fibrosis may contribute in part to this positive remodeling response. The net result of the increment in vessel circumference and the reduction in neointimal mass due to IPC paclitaxel is an increase in luminal size from C, 5.12±0.23 to HD, 6.15±0.25, and LD, 7.02±0.18 mm, ($p = 0.002$, 0.006 respectively). This dual effect of paclitaxel on remodeling and proliferation is encouraging since multiple studies have suggested that both increased total SMC bulk and vascular remodeling are key contributors to restenosis after angioplasty, while numerous therapeutic agents affecting predominantly SMCs proliferation have been found insufficient to prevent vessel renarrowing.

MMPs and their inhibitors, which regulate extracellular matrix homeostasis, might play a significant role in normal and pathologic vessel remodeling. Degradation of the

elastic laminae by MMP-2 is accentuated in both inward remodeling due to low flow and outward remodeling due to high flow, and appears to be a necessary component of any lasting structural modification of the vessel wall.

MMP-2 expression was clearly detectable in the C and LD groups following porcine coronary angioplasty, but was generally lost or reduced in the HD vessel segments. The mechanism of MMP-2 downregulation following exposure to paclitaxel at the 50 mg dose is not clear. The presence of MMP-2 immunoreactivity in the C and LD groups must be interpreted with caution because of the absence of data confirming zymogen activation and molar excess with respect to tissue metalloprotease inhibitor (TIMP) levels, a limitation of this study. Nevertheless, the absence of MMP-2 staining in the HD group clearly suggests the lack of such activity in these specimens. This, in turn, generates the hypothesis that the loss of MMP-2 is linked to the diminished outward remodeling found in the HD group.

IPC paclitaxel treatment at both selected doses does not appear to be causing overt damage to either the endothelial or medial layers. Endothelial regeneration was present to a nearly complete degree in all groups. Likewise, medial cell densities and areas were no different among the three groups.

The diminished outward remodeling in the 50 mg dosage group is largely responsible for partial loss of the anti-stenotic effect seen upon administration of the lower dose of drug. This biphasic dose-response may be interpreted as defining the transition into a supra-therapeutic drug level for this delivery modality.

CONCLUSIONS

A single-dose perivascular delivery of paclitaxel into the pericardial space significantly preserves luminal patency in the porcine coronary balloon overstretch model. The mechanism by which IPC delivery of paclitaxel maintains vascular lumen area involves promotion of positive vascular remodeling as well as inhibition of SMC hyperplasia. This study further establishes a maximum dose for IPC paclitaxel using the polymeric formulation described, and suggests that a carefully chosen dose of paclitaxel can be employed for the inhibition of post-angioplasty restenosis via IPC delivery.

Table 1. Histologic Changes in Pericardium 28 Days after Paclitaxel Delivery

	Adhesion score	Mesothelium Intact/Total	Visceral thickness (mm)
Control	0.20±0.20	5/5	0.22±0.02
Low-dose	0.33±0.21	6/6	0.23±0.03
High-dose	2.57±0.20*	3/7	0.45±0.02*

Values are Mean ± SEM.
Mesothelium indicates the number of pigs with mesothelium layer divided by each group total pigs.
* $p < .001$ versus control and low-dose.

Table 2. Morphometric Analysis of the Effect of IPC Paclitaxel Delivery

	Control	High-dose	Low-dose	*P*
FL/FL+IEL	0.21±0.02	0.22±0.03*	0.21±0.01*	* = NS
Intimal area (IA, mm^2)	0.79±0.07	0.51±0.06*	0.47±0.04*	* < .001
IA/FL	0.68±0.03	0.39±0.04*	0.32±0.02*°	* < .001; °.043
Maximal intima thickness(mm)	0.56±0.02	0.42±0.03*	0.43±0.03*	* < .001
Media area (MA, mm^2)**	1.07±0.07	1.15±0.10*	1.13±0.05*	* = NS
Maximal adventitial thickness(mm)	0.47±0.02	0.38±0.03*	0.35±0.02*	* < .001
External lamina circumference (mm)	7.04±0.22	7.71±0.23*	8.12±0.18*°	* = .001; °.017
External lamina area (mm^2)**	3.94±0.18	4.73±0.19*	5.25±0.20*°	* = .001; °.017
Endoluminal circumference (mm)	5.12±0.23	6.15±0.25*	7.02±0.18*°	* = .002; °.006

Values are Mean ± SEM.
FL indicates fracture length. IEL indicates internal elastic lamina length.
* versus control, ° low-dose versus high-dose.
** Area values for these parameters determined from circumference values using circular geometry.

EXAMPLE 13

TREATMENT OF RESTENOSIS IN RELATION TO VASCULAR GRAFT

MATERIALS AND METHODS:

The experiment involved implantation of a vascular graft between the common carotid artery and the external jugular vein in pigs (4 mm graft, n=26; 6 mm graft, n=19).

However, graft patency could not consistently be maintained due to a high frequency of graft thrombosis (75% in 4mm grafts and 50% in 6mm grafts). Thus, we developed a pig model of arterio-venous (AV) fistula where the end of the internal jugular vein was connected to the side of the common carotid artery (n=12). Pigs weighing 40 kg to 50 kg were anesthetized with 1.5% halothane after sedation with ketamine. A vertical incision was made on the right side of the trachea, and the right common carotid artery and internal jugular vein were exposed. Blood flow in the vein was interrupted proximally with a vascular clamp. The vein was tied off distally and cut. Blood flow in the artery was stopped with two vascular clamps and an arteriotomy made between the clamps. The free end of the vein was sutured to the artery with 6-0 Proline. Blood flow was restored by removing all three clamps. Bleeding at the anastomosis was controlled by temporary application of Gelfoam and patency of the fistula was checked. A 4 cm x 6 cm paclitaxel loaded (20% or 5%) EVA film or a control EVA film devoid of drug was applied around the anastomosis. The wound was closed. The same procedure was performed on the left side but the anastomosis was not treated so that each animal could be used as its own control. Two pigs were used in each group. After 28 days, the animals were sacrificed and pressure perfused at 100 mmHg with 10% buffered formaldehyde. Both AV fistulae were harvested. Cross-sections were cut within the anastomosis and in the artery and vein. Sections were stained with hematoxylin-and-eosin and Movat's stains and the effect of paclitaxel on venous stenosis was assessed.

RESULTS:

Untreated AV fistulae exhibited a pronounced venous intimal thickening (Figure 9B and 10B). The neointima exhibited numerous cells and the extracellular matrix was rich in collagen and proteoglycan. Intimal hyperplasia was inhibited in animals treated with 5% and 20% paclitaxel (Figure 9A and 10A). It was not affected by control EVA films devoid of paclitaxel. Similarly to Experiment I in rats, an acellular fibrin layer was present around AV fistulae treated with paclitaxel (Figure 9A and 10A).

CONCLUSION

Perivascular paclitaxel slowly released from EVA films prevents venous intimal hyperplasia after creation of an arterio-venous shunt. The presence of the

periadventitial fibrin layer suggests that the treated anastomoses are stronger than the control ones. This technology should extend the life of vascular grafts in humans by reducing venous stenosis, the principal cause of graft failure.

EXAMPLE 14

EFFECT OF ANTI-MICROTUBULE AGENTS ON NEUTROPHIL ACTIVITY

The example describes the effect of anti-microtubule agents on the response of neutrophils stimulated with opsonized CPPD crystals or opsonized zymosan. As shown by experiments set forth below, anti-microtubule agents are strong inhibitors of particulate-induced neutrophil activation as measured by chemiluminescence, superoxide anion production and degranulation in response to plasma opsonized microcrystals or zymosan.

MATERIALS AND METHODS

Hanks buffered saline solution (HBSS) pH 7.4 was used throughout this study. All chemicals were purchased from Sigma Chemical Co (St. Louis, MO) unless otherwise stated. All experiments were performed at 37°C unless otherwise stated.

1. PREPARATION AND CHARACTERIZATION OF CRYSTALS

CPPD (triclinic) crystals were prepared. The size distribution of the crystals was approximately 33% less than 10 μm, 58% between 10 and 20 μm and 9% greater than 20 μm. Crystals prepared under the above conditions are pyrogen-free and crystals produced under sterile, pyrogen-free conditions produced the same magnitude of neutrophil response as crystals prepared under normal, non-sterile laboratory conditions.

2. OPSONIZATION OF CRYSTALS AND ZYMOSAN

All experiments that studied neutrophil responses to crystals or zymosan in the presence of paclitaxel were performed using plasma opsonized CPPD or zymosan. Opsonization of crystals or zymosan was done with 50% heparinized plasma at a concentration of 75 mg of CPPD or 12 mg of zymosan per ml of 50% plasma. Crystals or zymosan were incubated with plasma for 30 minutes at 37°C and then washed in excess HBSS.

3. NEUTROPHIL PREPARATION

Neutrophils were prepared from freshly collected human citrated whole blood. Briefly, 400 ml of blood were mixed with 80 ml of 4% dextran T500 (Phamacia LKB, Biotechnology AB Uppsala, Sweden) in HBSS and allowed to settle for 1 hour. Plasma was collected continuously and 5 ml applied to 5 ml of Ficoll Paque (Pharmacia) in 15 ml polypropylene tubes (Corning, NY). Following centrifugation at 500 g for 30 minutes, the neutrophil pellets were washed free of erythrocytes by 20 seconds of hypotonic shock. Neutrophils were resuspended in HBSS, kept on ice and used for experiments within 3 hours. Neutrophil viability and purity was always greater than 90%.

4. INCUBATION OF NEUTROPHILS WITH ANTI-MICROTUBULE AGENTS

(a) Paclitaxel

A stock solution of paclitaxel at 12 mM in dimethylsulfoxide (DMSO) was freshly prepared before each experiment. This stock solution was diluted in DMSO to give solutions of paclitaxel in the 1 to 10 mM concentration range. Equal volumes of these diluted paclitaxel solutions was added to neutrophils at 5,000,000 cells per ml under mild vortexing to achieve concentrations of 0 to 50 μM with a final DMSO concentration of 0.5%. Cells were incubated for 20 minutes at 33°C then for 10 minutes at 37°C before addition to crystals or zymosan.

(b) Aluminum Fluoride

A stock solution of aluminum fluoride (AlF_3) at 1 M in HBSS was freshly prepared. This stock solution was diluted in HBSS to give solutions of AlF_3 in the 5 to 100 mM concentration range. Equal volumes (50 μl) of these diluted AlF_3 solutions was added to neutrophils at 5,000,000 cells per ml and incubated for 15 minutes at 37°C. Luminol (1 μM) was added and then 20 μl of opsonized zymosan (final concentration = 1 mg/ml) to activate the cells.

(c) Glycine Ethyl Ester

A stock solution of glycine ethyl ester at 100 mM in HBSS was freshly prepared. This stock solution was diluted in HBSS to give solutions of glycine ethyl ester in

the 0.5 to 10 mM concentration range. Equal volumes (50 µl) of these diluted glycine ethyl ester solutions was added to neutrophils at 5,000,000 cells per ml and incubated for 15 minutes at 37°C. Luminol (1 µM) was added and then 20 µl of opsonized zymosan (final concentration = 1 mg/ml) to activate the cells.

(d) LY290181

A stock solution of LY290181 at 100 µM in HBSS was freshly prepared. This stock solution was diluted in HBSS to give solutions of LY290181 in the 0.5 to 50 µM concentration range. Equal volumes (50 µl) of these diluted LY290181 solutions was added to neutrophils at 5,000,000 cells per ml and incubated for 15 minutes at 37°C. Luminol (1 µ M) was added and then 20 µl of opsonized zymosan (final concentration = 1 mg/ml) to activate the cells.

5. CHEMILUMINESCENCE ASSAY

All chemiluminescence studies were performed at a cell concentration of 5,000,000 cells/ml in HBSS with CPPD (50 mg/ml). In all experiments 0.5 ml of cells was added to 25 mg of CPPD or 0.5 mg of zymosan in 1.5 ml capped Eppendorf tubes. 10 µl of luminol dissolved in 25% DMSO in HBSS was added to a final concentration of 1 µM and the samples were mixed to initiate neutrophil activation by the crystals or zymosan. Chemiluminescence was monitored using an LKB Luminometer (Model 1250) at 37°C for 20 minutes with shaking immediately prior to measurements to resuspend the crystals or zymosan. Control tubes contained cells, drug and luminol (crystals absent).

6. SUPEROXIDE ANION GENERATION

Superoxide anion concentrations were measured using the superoxide dismutase inhibitable reduction of cytochrome C assay. Briefly, 25 mg of crystals or 0.5 mg of zymosan was placed in a 1.5 ml capped Eppendorf tube and warmed to 37°C. 0.5 ml of cells at 37°C were added together with ferricytochrome C (final concentration 1.2 mg/ml) and the cells were activated by shaking the capped tubes. At appropriate times tubes were centrifuged at 10,000g for 10 seconds and the supernatant collected for assay be measuring the absorbance of 550 nm. Control tubes were set up under the same conditions with the inclusion of superoxide dismutase at 600 units per ml.

7. NEUTROPHIL DEGRANULATION ASSAY

One and a half milliliter Eppendorf tubes containing either 25 mg of CPPD or 1 mg of zymosan were preheated to 37°C. 0.5 ml of cells at 37°C were added followed by vigorous shaking to initiate the reactions. At appropriate times, tubes were centrifuged at 10,000 g for 10 seconds and 0.4 ml of supernatant was stored at -20°C for later assay.

Lysozyme was measured by the decrease in absorbance at 450 nm of a *Micrococcus lysodeikticus* suspension. Briefly, *Micrococcus lysodeikticus* was suspended at 0.1 mg/ml in 65 mM potassium phosphate buffer, pH 6.2 and the absorbance at 450 nm was adjusted to 0.7 units by dilution. The crystal (or zymosan) and cell supernatant (100 µl) was added to 2.5 ml of the *Micrococcus* suspension and the decrease in absorbance was monitored. Lysozyme standards (chicken egg white) in the 0 to 2000 units/ml range were prepared and a calibration graph of lysozyme concentration against the rate of decrease in the absorbance at 450 nm was obtained.

Myeloperoxidase (MPO) activity was measured by the increase in absorbance at 450 nm that accompanies the oxidation of dianisidine. 7.8 mg of dianisidine was dissolved in 100 ml of 0.1 M citrate buffer, pH 5.5 at 3.2 mM by sonication. To a 1 ml cuvette, 0.89 ml of the dianisidine solution was added, followed by 50 µl of 1% Triton x 100, 10 µl of a 0.05% hydrogen peroxide in water solution and 50 µl of crystal-cell supernatant. MPO activity was determined from the change in absorbance (450 nm) per minute, Delta Å 450, using the following equation:

Dianisidine oxidation (nmol/min) = 50 x Delta Å 450

8. NEUTROPHIL VIABILITY

To determine the effect of the anti-microtubule agents on neutrophil viability the release of the cytoplasmic marker enzyme, lactate dehydrogenase (LDH) was measured. Control tubes containing cells with drug (crystals absent) from degranulation experiments were also assayed for LDH.

B. Results

In all experiments statistical significance was determined using Students' t-test and significance was claimed at $p<0.05$. Where error bars are shown they describe one

standard deviation about the mean value for the n number given.

1. NEUTROPHIL VIABILITY

(a) Paclitaxel

Neutrophils treated with paclitaxel at 46 μM for one hour at 37°C did not show any increased level of LDH release (always less than 5% of total) above controls indicating that paclitaxel did not cause cell death.

(b) Aluminum Fluoride

Neutrophils treated with aluminum fluoride at a 5 to 100 mM concentration range for 1 hour at 37°C did not show any increased level of LDH release above controls indicating that aluminum fluoride did not cause cell death.

(c) Glycine Ethyl Ester

Neutrophils treated with glycine ethyl ester at a 0.5 to 20 mM concentration range for 1 hour at 37°C did not show any increased level of LDH release above controls indicating that glycine ethyl ester did not cause cell death.

2. CHEMILUMINESCENCE

(a) Paclitaxel

Paclitaxel at 28 μM produced strong inhibition of both plasma opsonized CPPD and plasma opsonized zymosan-induced neutrophil chemiluminescence. The inhibition of the peak chemiluminescence response was 52% (+/-12%) and 45% (+/-11%) for CPPD and zymosan respectively. The inhibition by paclitaxel at 28 μM of both plasma opsonized CPPD and plasma opsonized zymosan-induced chemiluminescence was significant at all times from 3 to 16 minutes. These experiments show the concentration dependence of paclitaxel inhibition of plasma opsonized CPPD-induced neutrophil chemiluminescence. In all experiments control samples never produced chemiluminescence values of greater than 5 mV and the addition of paclitaxel at all concentrations used in this study had no effect on the chemiluminescence values of controls.

(b) Aluminum Fluoride

Aluminum fluoride at concentrations of 5 to 100 mM produced strong inhibition of plasma opsonized zymosan-induced neutrophil chemiluminescence, in a concentration dependent manner (concentration dependence of AlF_3 inhibition of plasma opsonized zymosan-induced neutrophil chemiluminescence). The addition of AlF_3 at all concentrations used in this study had no effect on the chemiluminescence values of controls.

(c) Glycine Ethyl Ester

Glycine ethyl ester at concentrations of 0.5 to 20 mM produced strong inhibition of plasma opsonized zymosan-induced neutrophil chemiluminescence (glycine ethyl ester inhibition of plasma opsonized zymosan-induced neutrophil chemiluminescence was concentration dependent). The addition of glycine ethyl ester at all concentrations used in this study had no effect on the chemiluminescence values of controls.

(d) LY290181

LY290181 at concentrations of 0.5 to 50 μM produced strong inhibition of plasma opsonized zymosan-induced neutrophil chemiluminescence. The addition of LY290181 at all concentrations used in this study had no effect on the chemiluminescence values of controls.

3. SUPEROXIDE GENERATION

Plasma opsonized CPPD crystal-induced superoxide anion production, as measured by the superoxide dismutase (SOD) inhibitable reduction of cytochrome C. In particular, treatment of the cells with paclitaxel at 28 μM produced a decrease in the amount of superoxide generated at all times. This decrease was significant at all times. Stimulation of superoxide anion production by opsonised zymosan showed a similar time course to CPPD-induced activation. The inhibition of zymosan-induced superoxide anion production by paclitaxel at 28 μM was less dramatic than the inhibition of CPPD activation but was significant at all times tested

Treatment of CPPD crystal-induced neutrophils with LY290181 at 17 μM also produced a decrease in the amount of superoxide generated.

4. NEUTROPHIL DEGRANULATION

Neutrophil degranulation was monitored by the plasma opsonized CPPD crystal-induced release of myeloperoxidase and lysozyme or the plasma opsonized zymosan-induced release of myeloperoxidase. It has been shown that sufficient amounts of these two enzymes are released into the extracellular media when plasma coated CPPD crystals are used to stimulate neutrophils without the need for the addition of cytochalasin B to the cells.

Paclitaxel at 28 μM reduced lysozyme release and this inhibition of degranulation was significant at all times tested.

Only minor amounts of MPO and lysozyme were released when neutrophils were stimulated with opsonized zymosan. Despite these low levels it was possible to monitor 50% inhibition of MPO release after 9 minutes incubation in the presence of paclitaxel at 28 μM that was statistically significant ($p<0.05$) (data not shown). Treatment of CPPD crystal-induced neutrophils with LY290181 at 17 μM decreased both lysozyme and myeloperoxidase release from the

C. Discussion

These experiments demonstrate that paclitaxel and other anti-microtubule agents are strong inhibitors of crystal-induced neutrophil activation. In addition, by showing similar levels of inhibition in neutrophil responses to another form of particulate activator, opsonized zymosan, it is evident that the inhibitory activity of paclitaxel and other anti-microtubule agents are not limited to neutrophil responses to crystals. Paclitaxel, aluminum fluoride, glycine ethyl ester and LY290181 were also shown to be strong inhibitors of zymosan-induced neutrophil activation without causing cell death. LY290181 was shown to decrease superoxide anion production and degranulation of CPDD crystal-induced neutrophils.

EXAMPLE 15

T CELL RESPONSE TO ANTIGENIC STIMULUS

In order to determine whether paclitaxel affects T-cell activation in response to

stimulagens, TR1 T-cell clones were stimulated with either the myelin basic protein peptide, GP68-88, or the lectin, conA, for 48 hours in the absence or presence of increasing concentrations of paclitaxel in a micellar formulation. Paclitaxel was added at the beginning of the experiment or 24 hours following the stimulation of cells with peptide or conA. Tritiated thymidine incorporation was determined as a measure of T-cell proliferation in response to peptide or conA stimulation.

The results demonstrated that T-cell stimulation increased in response to the peptide GP68-88 and conA. In the presence of control polymeric micelles, T-cell stimulation in response to both agonists was not altered. However, treatment with paclitaxel micelles, either at the beginning of the experiment or 24 hours following the stimulation, decreased T-cell response in a concentration dependent manner. Under both conditions, T-cell proliferation was completely inhibited by 0.02 μM paclitaxel

These data indicate that paclitaxel is a potent inhibitor of T-cell proliferation in response to antigen-induced stimulation.

From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the invention is not limited by the specific examples provided herein.

CLAIMS

1. A method for treating or preventing disease of the pericardium, heart, or coronary vasculature, comprising administering intrapericardially to a patient an anti-microtubule agent, such that said disease of the pericardium, heart, or coronary vasculature is treated or prevented.

2. The method according to claim 1 wherein said anti-microtubule agent is paclitaxel, or an analogue or derivative thereof.

3. The method according to claim 1 wherein said disease is intimal hyperplasia.

4. The method according to claim 1 wherein said anti-microtubule agent further comprises a polymer.

5. The method according to claim 1 wherein said polymer is poly-lactic acid.

6. The method according to claim 1 wherein said polymer is hyaluronic acid.

7. The method according to claim 5 wherein said biodegradable polymer is comprised of poly(hydroxy acid), poly (lactones), poly (amino acids), poly (anhydrides), poly (orthoesters), poly (phosphazines), poly (phosphesters), poly saccharrides, and the co-polymers and blends of any of these.

8. The method according to claim 1 wherein said polymer is poly-lactic acid.

9. The method according to claim 1 wherein said polymer is hyaluronic

acid.

10. The method according to claim 1 wherein said disease is stenosis, restenosis, or in-stent restenosis.

11. The method according to claim 1 wherein said disease is atherosclerosis.

12. The method according to claim 1 wherein said disease is transplant rejection.

13. The method according to claim 1 wherein said disease is arteritis.

14. The method according to claim 1 wherein said disease is a rheumatic condition affecting the heart.

15. The method according to claim 1 wherein said disease is valvular stenosis.

16. The method according to claim 1 wherein said disease is shunt restenosis.

17. The method according to claim 1 wherein said disease is cardiac adhesion.

18. The method according to claim 1 wherein said disease is a malignant pericardial effusion.

19. The method according to claim 1 wherein said disease is a cardiac rhythm disorder.

Fig. 1

Intrapericardial Micellar Paclitaxel - Treatment Protocol

WO 00/44443 PCT/US00/02376

Fracture Length 28 Days Following Balloon Injury in Swine Coronary Arteries

Fig. 2

SUBSTITUTE SHEET (RULE 26)

Intrapericardial Micellar Paclitaxel Reduces Calculated Diameter Coronary Stenosis

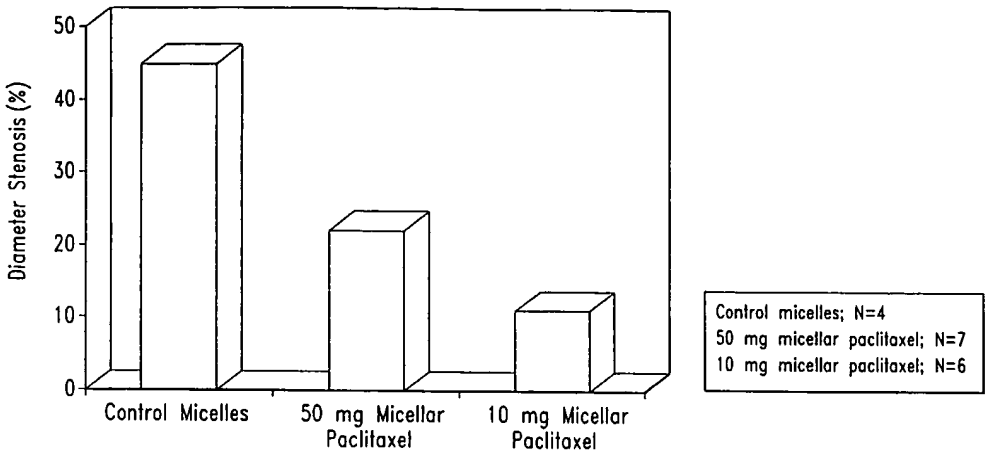
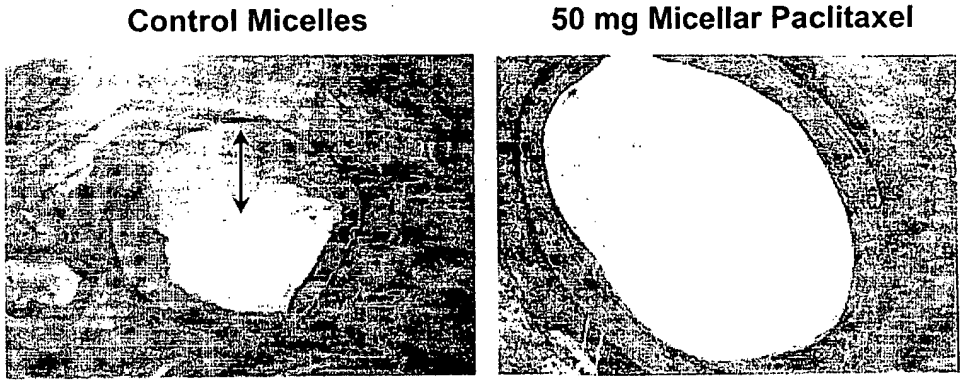


Fig. 3

Fig. 4

Intrapericardial Micellar Paclitaxel
(Swine Coronary Artery Balloon Injury Model)



Intrapericardial Micellar Paclitaxel Results in Significantly Reduced Neointimal Area

Fig. 5

Intrapericardial Micellar Paclitaxel Treatment Results in Increased Histologic Lumen Diameter

Fig. 6

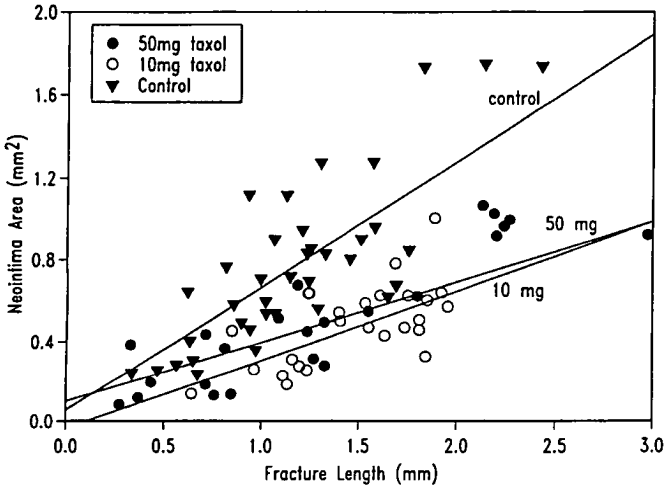


Fig. 7

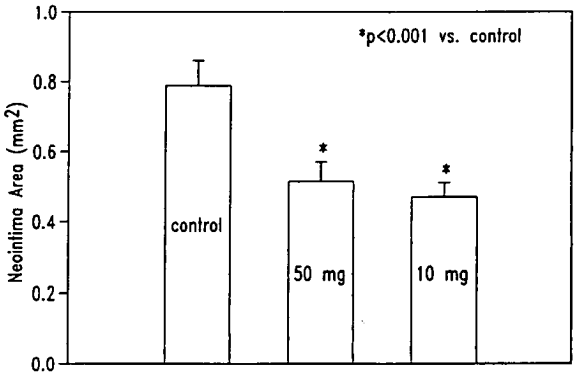


Fig. 8A

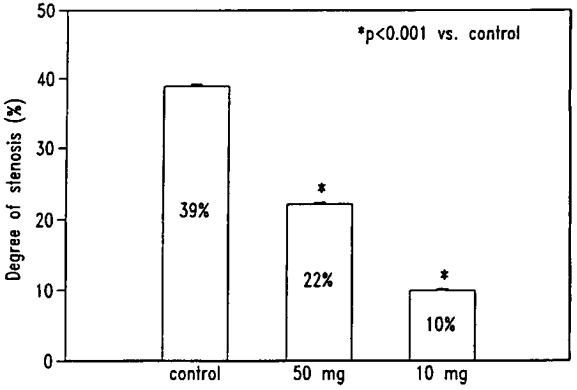


Fig. 8B

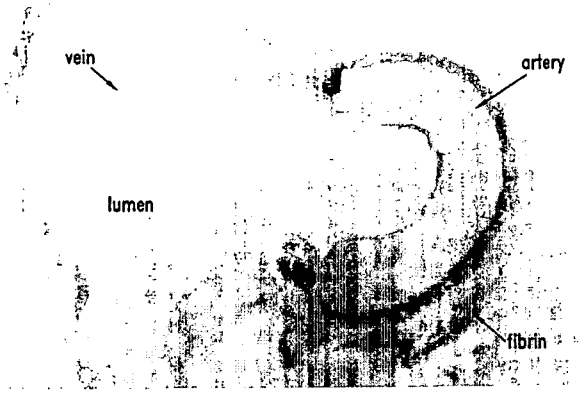


Fig. 9A

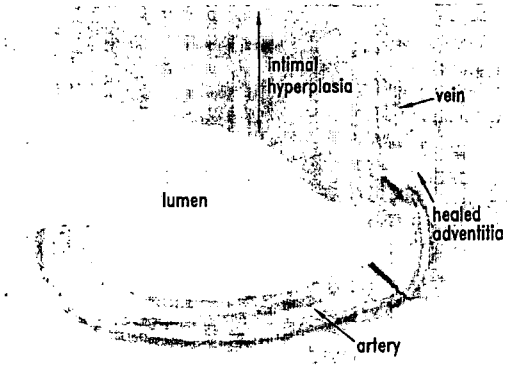


Fig. 9B

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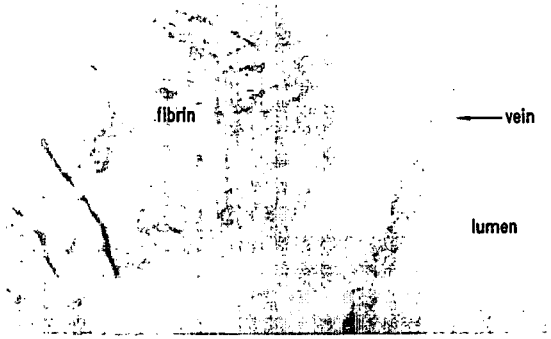

Fig. 10A

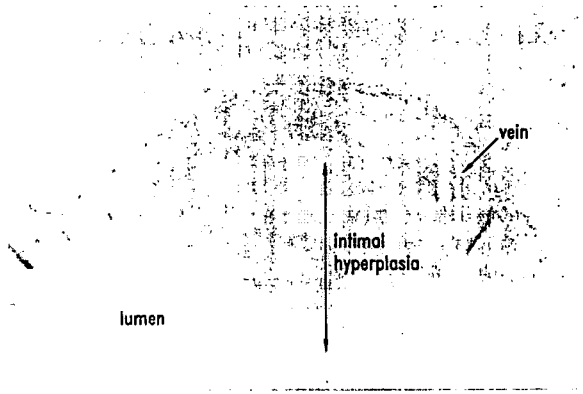

Fig. 10B

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/02376

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/337 A61P9/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, CHEM ABS Data, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SUH H ET AL: "Regulation of smooth muscle cell proliferation using paclitaxel-loaded poly(ethylene oxide)-poly(lactide/glycolide) nanospheres." JOURNAL OF BIOMEDICAL MATERIALS RESEARCH, (1998 NOV) 42 (2) 331-8., XP000937874 the whole document ---	1-10,15, 16
X	WO 97 33552 A (LI CHUN ;WALLACE SIDNEY (US); YU DONG FANG (US); WALLACE TECH INC) 18 September 1997 (1997-09-18) page 9, line 11 -page 10, line 5 page 15, line 13-27 claims 1,12,13,36-40; examples 2,3 --- -/--	1-5,7,8, 10,15,16

[X] Further documents are listed in the continuation of box C. [X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 September 2000	23.03.01
Name and mailing address of the ISA: European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer: Veronese, A

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/02376

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 25176 A (RENO JOHN M ;KUNZ LAWRENCE L (US); NEORX CORP (US)) 22 August 1996 (1996-08-22) claims 1,2,4,8,9,11,18-22,27-30,36-43,49-51,53-55 claims 57-59,61-66 ---	1-4,10, 15,16
X	WO 98 43618 A (NEORX CORP) 8 October 1998 (1998-10-08) claims 1,8-23,28-36,56-59,62-64,74-77 ---	1-3,10, 15,16
X	SOLLOTT ET AL: "Taxol inhibits neointimal smooth muscle cell accumulation after angioplasty in the rat" JOURNAL OF CLINICAL INVESTIGATION,US,NEW YORK, NY, vol. 4, no. 95, 1 April 1995 (1995-04-01), pages 1869-1876, XP002075727 ISSN: 0021-9738 page 1875, column 1, line 32-41 ---	1-4,10, 15,16
X	KOMOWSKI R ET AL: "SLOW-RELEASE TAXOL COATED GRIITM STENTS REDUCE NEOINTIMA FORMATION IN A PORCINE CORONARY IN-STENT RESTENOSIS MODEL" CIRCULATION,US,AMERICAN HEART ASSOCIATION, DALLAS, TX, vol. 96, no. 8, 1997, page 1341 XP000891206 ISSN: 0009-7322 the whole document ---	1-4,10, 15,16
X	KUNERT ET AL: "Paclitaxel inhibits development of restenosis following experimental ballon angioplasty in the rabbit carotid artery" EUROPEAN HEART JOURNAL,XX,THE EUROPEAN SOCIETY OF CARDIOLOGY, no. 17, 1996, page 368 XP002075724 ISSN: 0195-668X the whole document ---	1-3,10, 15,16
X	WO 95 03795 A (US ARMY ;KINSELLA JAMES L (US); SOLLOTT STEVEN J (US)) 9 February 1995 (1995-02-09) claims 1,7-9,12-16,20-24 ---	1-3,10, 15,16
E	WO 00 41687 A (ALVARADO ANGELICA ;QUANAM MEDICAL CORP (US); EURY ROBERT (US); FRO) 20 July 2000 (2000-07-20) the whole document --- -/--	1-4,10, 15,16

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/02376

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	AXEL D I ET AL: "Paclitaxel inhibits arterial smooth muscle cell proliferation and migration in vitro and in vivo using local drug delivery [see comments]." CIRCULATION, (1997 JUL 15) 96 (2) 636-45., XP000937700 the whole document ---	1-10,15, 16
Y	ZHANG, XICHEN ET AL: "Development of biodegradable polymeric paste formulations for taxol: an in vitro and in vivo study" INT. J. PHARM. (1996), 137(2), 199-208, XP000938230 the whole document ---	1-10,15, 16
P,Y	BURT, HELEN M. ET AL: "Development of copolymers of poly(DL-lactide) and methoxypolyethylene glycol as micellar carriers of paclitaxel" COLLOIDS SURF., B (1999), 16(1-4), 161-171, XP000937878 the whole document ---	1-10,15, 16
Y	ZHANG, XICHEN ET AL: "An investigation of the antitumour activity and biodistribution of polymeric micellar paclitaxel." CANCER CHEMOTHERAPY AND PHARMACOLOGY, (1997) VOL. 40, NO. 1, PP. 81-86., XP000937843 the whole document -----	1-10,15, 16

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 00/02376

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-9 (partial); 10, 15, 16 (complete)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (1)) (July 1998)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: Claims 1-9, (partial); 10,15,16 (complete).

   Pharmaceutical compositions for intra pericardial administration comprising paclitaxel, in relation to the treatment of stenosis, restenosis, stent-stenosis, valvular stenosis, shunt restenosis.

2. Claims: Claims 1-9, (partial); 11(complete).

   Pharmaceutical compositions for intra pericardial administration comprising paclitaxel, in relation to the treatment of atherosclerosis.

3. Claims: Claims 1-9, (partial); 12 (complete).

   Pharmaceutical compositions for intra pericardial administration comprising paclitaxel, in relation to the treatment of transplant rejection.

4. Claims: Claims 1-9, (partial); 13 (complete).

   Pharmaceutical compositions for intra pericardial administration comprising paclitaxel, in relation to the treatment of arteritis.

5. Claims: Claims 1-9, (partial); 14 (complete).

   Pharmaceutical compositions for intra pericardial administration comprising paclitaxel, in relation to the treatment of a rheumatic condition.

6. Claims: Claims 1-9, (partial); 17 (complete).

   Pharmaceutical compositions for intra pericardial administration comprising paclitaxel, in relation to the treatment of cardiac adhesion.

7. Claims: Claims 1-9, (partial); 18 (complete).

   Pharmaceutical compositions for intra pericardial administration comprising paclitaxel, in relation to the treatment of malignant pericardial effusion.

8. Claims: Claims 1-9, (partial); 19 (complete).

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Pharmaceutical compositions for intra pericardial administration comprising paclitaxel, in relation to the treatment of cardiac rhythm disorders.

International Application No. PCT/US 00 02376

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ ~~206~~ 210

Continuation of Box 3.

Although claims 1-19 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Further defect(s) under Article 17(2)(a):

Continuation of Box 3.

The wording of claim 1 "an antimicrotubule agent" relates to compounds/compositions defined by reference to a desirable characteristic or property, namely the property of interacting and adversely affecting the microtubule behaviour.
The claims cover all compounds/compositions having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for only a very limited number of such compounds/compositions. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the compounds/compositions by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search for the first invention has been carried out for those parts of the claim which appear to be clear, concise, supported and disclosed, namely for the antimicrotubule agent "paclitaxel", related compounds having the skeleton of paclitaxel, with due regard to the general idea underlying the application.
N.B. there is a mistake in the numbering of the claims: claim 7 can not depend from claim 5 and claim 8 and 9 are identical to claim 5 and 6.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte. . .ional Application No
PCT/US 00/02376

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 9733552	A	18-09-1997	AU	2580697 A	01-10-1997
			BR	9710646 A	11-01-2000
			CA	2250295 A	18-09-1997
			CN	1217662 A	26-05-1999
			CZ	9802908 A	14-07-1999
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			PL	328807 A	15-02-1999
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